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EXPERIMENTAL METHODS AND THE PROBABLE ERROR IN FIELD EXPERIMENTS WITH SORGHUM[1]

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INTRODUCTION

THE FIELD STATION AT CHILLICOTHE, TEX.

In 1915 the location of the Chillicothe (Tex.) Field Station was changed from a small rented tract adjoining the town site of Chillicothe on the northwest to a larger field 4½ miles south and 1 mile west of the city. The land and buildings thereon were purchased by the Texas Agricultural Experiment Station in 1916 and formally incorporated in its organization as Texas Substation No. 12. The forage-crop experiments carried on at Chillicothe are all conducted under a cooperative agreement between the Office of Forage Crops, Bureau of Plant Industry, United States Department of Agriculture, and the Texas Agricultural Experiment Station.

Approximately 100 acres of land are included in the station boundaries, and this land is unusually level, although it has a gradual slope from the southeast to the northwest. (Fig. 1.) This natural drainage, a fall of approximately 16 feet in 188 rods, has been controlled by ditches so that it is carried almost due west directly through the experimental tract by a large ditch banked slightly on the north side. To catch the drainage water from the field south of the station, a smaller ditch has been constructed along the south border for about three-fourths the distance from the west side. The building site and pasture lots occupy about 14 acres in the southeastern corner of the station tract, and the remainder of the land has been divided into blocks 20 rods square, except for strips of land on the north, south, and west sides, as indicated in Figure 1. Separating the blocks are roadways 20 feet wide, except for the main drive. These square blocks were laid off thus to allow for a change in the direction of the rows and plots each year. Most of the experiments are with crops grown in cultivated rows, 40 inches apart, such as sorghum, cotton, and cowpeas, and the plots are approximately twelve times as long as they are wide. By having the direction of the plots north and south one year and east and west the following year, the effect of alleyways between plots is overcome. The general arrangement of the land for experimental purposes is illustrated in Figure 1, a topographical chart which also indicates the elevations at different points.

[1] Received for publication Aug. 6, 1928; issued December, 1928.

The soil is a dark, sandy loam which from general observation appears more uniform than the average experimental tract of this size. It lies entirely on the rolling prairie at an altitude of 1,406 feet. The surface soils are derived from the Permian formation and belong mostly to the Vernon and Kirkland series. Certain small areas, the surfaces of which had been removed in leveling the land, thus bringing the calcareous strata nearer the surface, were mapped as Hardeman soils.

A somewhat detailed soil survey of the station tract was made in 1919.[2] The results of this survey are indicated in Figure 2. The experiment described in this paper was located in block G2 on the west side of the station tract, situated in 1915 as indicated by the hatched area on the soil map. In block G2 as then located only soils of the Kirkland series are found. Most of the area is occupied by

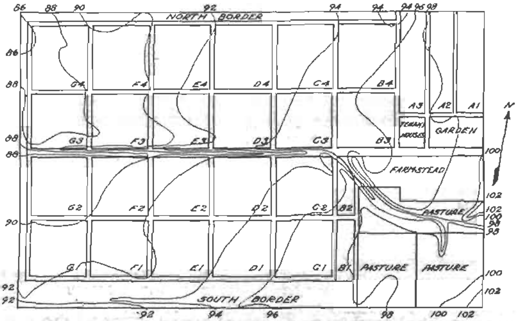

FIG. 1.—Topographical chart of Texas Substation No. 12, Chillicothe, Tex., showing contour lines and divisions of the area for experimental purposes

Kirkland loam, the surface 6 to 8 inches of which is a dark-browy friable loam which assumes a grayish color on top when it is dry. The subsoil down to 24 inches is a dark-brown or brown friable clay. From 24 to 36 inches of the subsoil is a grayish brown slightly calcareous clay which at 36 inches is nearly yellow or has yellow spots in it.

Kirkland clay loam differs from the Kirkland loam mainly in that the surface soil is a clay loam or silty clay loam and in some places is only 4 inches deep. The subsoil is about the same as that of the first type. Kirkland silt loam, as the name indicates, has a friable silt loam for the surface 8 inches. This surface soil grades into a silty clay loam beneath and at 16 to 20 inches becomes much more compact. At 20 inches it is grayish brown speckled with white, gray, and brown clay, and the subsoil is quite calcareous. The Kirkland fine sandy loam is found on the lower levels and is different from the loam chiefly because erosion from higher levels has left a deposit of

[2] This survey was carried out under the direction of W. T. Carter, Bureau of Chemistry and Soils, U. S. Department of Agriculture, for the Texas Agricultural Experiment Station, by T. M. Bushnell, A. C. Anderson, and Neal Gearreald.

fine sand on it, and this has been mixed with the surface 6 to 8 inches through cultivation.

The land on which the station is located had been farmed for a good many years, very largely to uniform crops of wheat, with only occasional crops of cotton. Although not so productive as some of the soils in northern Texas, this land was of at least average fertility, lacking chiefly in humus. When the land was taken over it was decided to follow a rotation consisting of small grain (wheat or oats), sorghum, cotton, and cowpeas. This had the advantage of ridding the soil of volunteer plants and (as much of the cowpea crop was plowed under) of adding a certain amount of humus to the soil. Owing to the pressure for an increased acreage of land for the cotton experi-

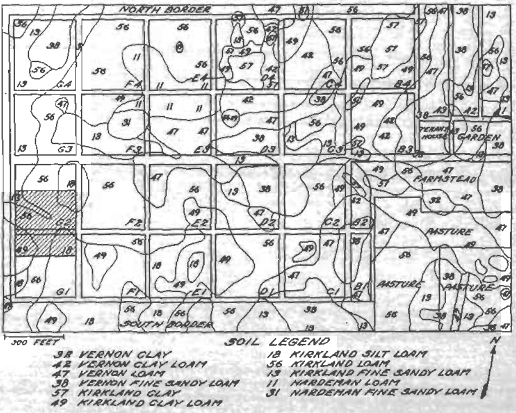

FIG. 2.—Soil map of Texas Substation No. 12, showing soil types and the location of experimental area G2 as situated in 1915

ments and the small importance of the cowpea crop in this section, it has not been found possible to continue this rotation in its entirety. The results indicate, however, that the productiveness of the soil and its uniformity as a whole have increased under this method of handling the experiments. The strips of land on the north and south sides and the blocks north of the building site are used for miscellaneous experiments which do not fit into the general rotation. A tract of about 12 acres south of the building site, except for gardens and a small orchard, is devoted to pasture for the work stock and milk cows.

PURPOSE OF THE EXPERIMENT

The removal of the station to a new location provided an opportunity and also a reason for an experiment to determine the extent of error which might be expected on this land because of the soil heterogeneity. Since sorghum was destined to be one of the chief

crops in the work at Chillicothe, it was used as the indicator crop. A very uniform strain of feterita which had been selected for six years was seeded over an entire block in rows 40 inches apart and given the same cultural treatment as that accorded to the regular experiments. In seeding this block to feterita (F. C. 811), the chief thought was to obtain an increased quantity of the seed, and it was not until later in the year, as harvest time approached, that the idea developed of using this 2½ acres of feterita for the purpose of estimating the experimental error to be expected in future tests of sorghum. No extra precautions, such as replanting portions of the rows where vacancies in stand occurred, were taken. It is recognized that in any well-conducted experiment the number of unknown factors should be limited, if possible, to one. The fact remains, however, that in ordinary field experiments very few are so handled as to exclude completely little discrepancies of this sort. The results cover, therefore, not only differences due to soil heterogeneity, but also others due to imperfections in the stand such as appear in the average field experiment plot. That such imperfections were less, or at least no greater, in this block of feterita than in the better conducted field experiments, was the judgment of several who observed it closely before it was harvested. For the purpose of measuring the error that must be taken into consideration when conducting varietal or cultural experiments on the Chillicothe station farm, the results obtained are perhaps more useful than if unusual care had been taken to eliminate all effects on the yield other than those due to soil differences.

LOCATION OF THE EXPERIMENT

This feterita was grown on block G2, in the southwest corner of the station tract. The location of this block was about 8 rods farther south than at present. (See fig. 2 for present location.) The change was necessitated by drainage requirements which altered the location of the main ditch to provide for it directly adjacent to and parallel with the main drive through the experimental field from the entrance on the east to the west boundary. This drive and the ditch together occupy a strip of land about 40 feet wide through the middle of the station tract and withhold from experimental uses approximately 2½ acres of land. No better way of handling the surface water could be devised, however, because heavy torrential rains sometimes occur, and small shallow ditches overflow and allow sand and silt to be deposited on the experimental areas. An occurrence of this kind early in 1915 destroyed the uniformity of blocks G3 and G4.

Block G2, on which the feterita was grown, while not the most uniform area on the station, was no doubt of at least average uniformity. It had grown wheat the previous year and was therefore subjected to uniform culture processes. It is exceptionally level and has no drainage course through it.

CLIMATIC FEATURES OF 1915

Although not of any great importance in an experiment of this kind, since all the plants in the block are subjected to the same climatic conditions whether they are good or bad, it is perhaps worth while to indicate briefly the general seasonal conditions.

The total annual rainfall in 1915 was 34.81 inches, 8.8 inches above a 21-year average. The total for the growing season, May to September, inclusive, was 20.5 inches, 5.6 inches more than normal. The mean temperatures for the same months were: May, 68° F.; June, 78°; July, 81°; August, 76°; September, 75°. The lack of any effective rain from June 6 to July 19 resulted in a drought during July which reduced the yields somewhat.

CULTURAL METHODS

The ground was plowed and harrowed during January and February. It was disked and marked off in rows 40 inches apart preparatory to seeding in late May. The seed was sown with a drill, and it germinated to a fair stand. Very little thinning was required, and a count of the plants showed an average of 616 plants per row, which indicated an average row space of a trifle over 6 inches to each plant. The stand was estimated to be 90 per cent uniform, with only occasional small breaks. Only ordinary cultivation was given the crop, but when mature it was comparatively free from weeds, and the soil was at all times in good condition for growth.

METHODS OF HARVESTING

At harvest time the average height of the stems was 55 inches and the diameter five-eighths inch. R. W. Edwards, then superintendent of the station, reported observations as follows: "The west side of the block was not so good as the east side. [This proved true. Compare mean yields of "east third" and "west third," Figure 6.—H. N. V.] A large number of sucker heads were formed, most of which matured." The rows were marked off in rod lengths and harvested by hand. Weights of the green forage from each separate rod were obtained immediately as it was cut. The weights presented in this paper, therefore, represent green material. The yield was approximately 4.5 tons per acre, equivalent to 1.8 tons air-dry forage, a normal yield for this dwarf and rather fine-stemmed feterita.

The intention was to incorporate the data thus obtained in a publication on experimental methods at once, and the weights were reduced to ounces and the row totals calculated in the winter of 1915–16. Other more pressing work intervened, however, and delayed the completion of the task until the services of Mr. Stephens were obtained in 1926. No attempt has been made to review other publications on this subject. None has been presented which deals with the forage yields of sorghum grown in cultivated rows, and this contribution is made with the thought that it may serve to stimulate a desire to employ more accurate methods in experiments with such crops.

FIELD EXPERIMENTS

Original yields of the feterita are given in Table 1. No new methods have been introduced in handling the data, and the procedure is subject to whatever criticism may properly be directed at the usual ways of conducting blank experiments. Several plans advanced for reducing the error in field experiments have not been applied to the data here.

TABLE 1.—*Experimental-error data in the sorghum experiment at Chillicothe, Tex., in 1915*

Row	Yield (ounces) of 1-rod portions of the rows of feterita in the order in which they occur in the field (facing east)																				Row
1	226	222	236	262	208	213	200	204	184	230	206	196	194	220	158	160	200	152	186	145	1
2	236	178	204	234	268	202	190	205	186	210	168	160	201	194	232	160	210	250	214	176	2
3	224	202	210	262	210	191	190	179	184	218	175	160	191	220	194	190	236	200	204	112	3
4	240	196	264	252	216	240	208	168	152	180	176	200	200	181	216	176	204	212	212	166	4
5	216	233	214	208	244	241	210	211	230	168	190	142	192	188	176	184	160	196	193	160	5
6	224	222	198	185	183	180	195	184	159	204	168	168	216	250	224	200	242	202	140	190	6
7	236	272	270	228	244	214	202	222	208	204	212	146	182	224	182	200	209	215	182	190	7
8	198	177	228	202	174	218	201	232	138	173	158	142	232	220	216	180	224	190	212	198	8
9	202	174	210	192	192	222	191	164	170	170	174	128	172	212	214	140	202	210	198	202	9
10	210	190	200	232	208	218	262	226	137	174	182	168	184	186	196	184	208	200	176	234	10
11	234	180	168	200	192	196	190	194	186	160	196	162	196	198	158	168	212	208	184	188	11
12	222	166	214	210	188	240	220	190	194	142	172	146	198	208	208	150	208	230	164	212	12
13	182	204	228	228	216	218	232	214	188	170	190	138	208	182	210	220	216	204	174	214	13
14	294	200	216	185	218	172	194	200	162	172	182	177	204	198	164	155	201	214	176	184	14
15	230	228	220	230	228	208	220	187	144	162	176	168	232	212	212	218	108	182	216	190	15
16	244	198	216	257	176	198	234	214	176	180	195	158	202	188	152	138	193	196	174	226	16
17	188	172	222	200	234	225	242	208	185	150	200	156	196	208	183	192	220	164	202	186	17
18	204	167	206	160	178	184	200	154	176	144	131	113	174	196	171	150	162	201	188	200	18
19	212	232	220	248	238	254	210	244	196	212	188	160	174	188	186	186	192	218	190	212	19
20	201	176	168	188	122	152	177	192	146	192	152	149	158	182	182	158	222	208	190	188	20
21	202	166	184	216	222	162	188	166	170	148	162	166	154	174	172	154	238	228	228	190	21
22	172	180	188	120	196	226	182	170	158	198	176	148	158	144	158	160	214	224	178	182	22
23	274	166	182	216	178	224	182	150	158	162	150	150	162	172	176	160	224	212	217	192	23
24	184	144	202	192	150	184	180	192	126	174	154	144	152	166	162	168	200	174	192	164	24
25	232	178	188	242	224	210	184	176	184	138	208	182	116	184	185	148	180	206	202	176	25
26	182	184	196	172	168	128	164	162	164	134	114	104	154	188	192	140	163	178	194	168	26
27	194	196	166	228	200	164	180	160	116	148	168	158	144	162	166	140	192	174	202	180	27
28	166	182	164	166	216	216	208	194	140	170	118	154	168	208	160	150	190	214	168	158	28
29	206	126	159	172	192	148	202	212	153	130	140	148	157	183	122	164	166	210	209	143	29
30	196	140	145	127	216	172	193	181	193	190	129	120	158	161	170	146	210	187	170	178	30
31	193	200	175	170	192	177	187	200	204	241	149	167	186	218	197	198	193	194	168	151	31
32	204	148	201	165	186	181	185	161	163	168	188	172	162	200	160	173	169	189	185	147	32
33	185	186	153	154	168	134	126	181	147	135	163	145	173	173	161	178	186	174	176	140	33
34	200	208	200	186	211	186	193	169	177	142	188	148	161	188	202	198	178	172	176	180	34
35	208	170	189	153	200	176	185	213	165	161	151	201	193	136	180	153	206	144	201	104	35
36	185	150	150	169	178	167	186	192	161	167	182	132	145	148	110	122	162	174	153	163	36
37	210	180	208	225	213	239	183	237	167	202	204	249	206	202	209	292	183	185	196	168	37
38	164	188	176	184	185	166	151	173	167	170	197	180	198	200	156	161	151	143	129	143	38
39	182	185	207	219	159	144	207	208	123	117	208	200	175	170	128	170	144	165	148	154	39
40	243	214	225	144	151	194	200	206	191	199	223	169	208	181	149	179	141	138	187	169	40
41	212	165	165	212	134	175	184	174	128	194	190	200	180	159	172	144	134	190	162	155	41
42	196	206	149	196	173	184	177	174	173	170	168	169	176	121	156	172	163	150	160	167	42
43	192	209	215	158	202	202	197	182	192	202	197	193	162	183	207	177	162	151	165	192	43
44	203	191	164	158	159	168	169	184	167	179	177	157	165	145	175	179	145	159	131	138	44
45	174	175	208	205	184	178	183	208	161	165	179	184	173	181	218	203	180	126	172	177	45
46	216	192	202	183	167	182	209	186	160	184	204	157	169	162	221	170	182	152	164	196	46
47	178	159	218	192	149	183	137	158	125	213	155	172	169	189	200	198	173	137	176	148	47
48	229	197	171	175	202	159	161	149	91	148	150	164	188	167	176	128	136	139	116	140	48

49	242	185	199	198	204	185	191	138	189	191	169	173	193	245	156	191	190	164	146	175	49
50	193	172	195	147	173	167	156	129	146	172	191	142	189	163	178	185	193	161	150	141	50
51	246	131	205	208	176	184	150	143	162	146	158	158	196	192	204	160	153	150	144	181	51
52	216	185	189	188	205	184	173	161	162	147	166	156	173	232	182	200	185	188	154	198	52
53	165	153	205	193	201	172	125	180	142	162	120	172	187	186	167	178	204	136	136	176	53
54	203	167	198	182	214	184	140	179	153	203	197	141	201	191	236	196	169	165	200	168	54
55	200	217	188	233	247	218	186	221	201	161	155	192	188	186	197	199	197	176	175	147	55
56	193	229	224	170	190	168	154	179	217	212	193	211	232	227	215	216	173	216	188	217	56
57	220	249	195	189	246	161	199	212	179	188	225	210	176	213	165	189	173	202	202	164	57
58	212	202	232	224	186	176	162	195	168	208	199	216	219	200	183	199	165	154	184	174	58
59	227	234	195	255	240	169	217	215	206	235	193	207	244	192	156	199	183	197	169	134	59
60	177	154	179	208	173	143	169	143	149	161	175	173	189	171	180	204	174	155	192	161	60
61	175	252	229	250	215	129	180	200	188	173	198	198	236	222	217	198	187	193	222	201	61
62	148	167	150	182	176	139	161	187	154	194	181	302	143	214	218	194	187	166	183	196	62
63	165	227	185	184	135	166	188	202	179	206	177	193	213	186	183	165	215	200	170	190	63
64	206	160	170	178	160	168	185	205	140	132	188	161	214	210	201	194	216	175	193	172	64
65	216	182	179	187	172	194	186	223	177	212	185	222	214	184	172	163	194	207	186	153	65
66	184	203	155	128	121	172	160	144	195	180	191	212	221	187	180	163	179	167	206	160	66
67	234	213	163	155	176	160	191	198	207	225	184	194	227	119	182	181	213	205	188	185	67
68	180	156	152	98	147	152	171	172	190	167	201	224	237	209	212	178	180	258	75	191	68
69	179	176	142	99	146	132	187	198	229	195	203	238	250	201	178	203	180	209	214	160	69
70	214	187	153	167	167	200	179	168	173	188	206	222	257	212	222	196	181	213	214	161	70
71	205	180	160	131	151	124	193	190	163	170	129	211	222	203	222	208	181	184	196	165	71
72	165	170	188	179	183	136	186	136	147	166	170	204	199	192	186	177	175	144	198	170	72
73	158	179	147	152	175	190	154	175	169	191	212	260	132	169	202	207	195	179	194	177	73
74	189	159	156	142	172	172	156	193	142	183	210	204	176	179	199	184	132	164	183	163	74
75	190	203	152	165	144	167	153	178	182	176	173	184	176	181	150	191	186	194	197	160	75
76	193	141	186	177	177	149	168	177	199	199	174	181	194	158	189	184	172	186	174	174	76
77	165	126	141	131	126	147	188	191	216	208	213	197	164	156	158	178	192	178	132	163	77
78	156	144	138	147	136	155	162	163	188	226	188	144	157	196	150	177	155	136	193	163	78
79	170	132	193	173	170	161	204	157	194	192	190	188	156	200	176	184	181	177	152	168	79
80	144	145	120	158	136	170	156	230	191	150	172	162	160	162	134	184	185	170	149	157	80
81	164	152	187	136	137	156	149	181	177	170	178	141	143	148	156	162	165	160	170	148	81
82	180	153	210	153	148	121	169	209	187	208	187	182	173	182	161	179	208	198	180	166	82
83	153	160	205	139	166	171	184	173	152	145	161	166	123	140	119	116	168	143	150	148	83
84	154	193	162	166	152	145	162	205	182	184	196	198	175	162	149	194	170	140	168	198	84
85	153	203	196	138	163	135	163	170	197	164	184	231	203	190	104	141	169	195	176	197	85
86	126	148	122	162	145	107	170	181	202	178	135	127	176	130	162	195	177	144	178	184	86
87	180	144	162	163	157	194	140	170	170	158	159	160	175	186	113	138	178	178	229	154	87
88	152	147	148	166	162	171	143	140	171	178	171	153	172	127	152	187	220	162	194	198	88
89	161	130	132	152	190	136	164	148	154	138	134	144	161	190	135	165	155	183	140	180	89
90	144	146	114	144	149	139	160	170	159	137	183	113	184	176	143	136	151	144	144	161	90
91	183	203	157	144	147	151	184	176	205	201	174	216	174	184	181	209	178	200	180	176	91
92	158	150	176	188	156	190	152	224	191	185	165	142	177	141	191	196	171	160	185	208	92
93	161	176	175	180	192	176	205	167	191	164	102	171	176	145	160	163	172	170	179	164	93
94	183	207	205	239	196	122	172	236	185	197	166	178	162	156	172	188	177	176	192	169	94
95	172	165	176	158	172	161	165	192	161	198	218	156	185	156	156	146	128	150	163	104	95
96	180	132	167	165	175	172	189	177	157	163	182	191	124	132	167	174	141	152	176	166	96
97	176	214	207	223	224	226	164	214	205	202	240	203	231	188	202	192	214	190	187	176	97
98	208	170	201	170	201	146	167	172	209	180	185	133	158	165	165	199	200	224	213	191	98
99	187	221	218	185	211	195	188	177	202	170	176	152	233	187	199	200	167	196	180	166	99
100	278	180	261	192	252	209	217	206	224	199	228	214	191	185	174	213	192	218	201	233	100

The unit area of 1-rod row 40 inches wide in which this block of feterita was harvested is 1/792 of an acre. The ultimate or rod-row plot is called 1/800 acre, and combination plots are multiples of 1/800 instead of the actual fraction. The difference is insignificant, and the fractions used are easier to follow. Thus, the largest combination plot is 160/396, but is is called 160/400 or 2/5 acre.

The block was 20 rods long and 100 rows wide, therefore there were 2,000 ultimate plots. The two outside rows on each side were discarded, which left 1,920 plots to be used in the calculations. These rows were discarded on the basis of the following border effect:

Row 1 plus 100, average yield, 14.34 per cent above mean of inside 98 rows.
Row 2 plus 99, average yield, 9.22 per cent above mean of inside 96 rows.
Row 3 plus 98, average yield, 5.33 per cent above mean of inside 94 rows.

The probable error of a single two-row plot (adjacent rows and using all rows) is 4.53 per cent of the mean. The two outside rows

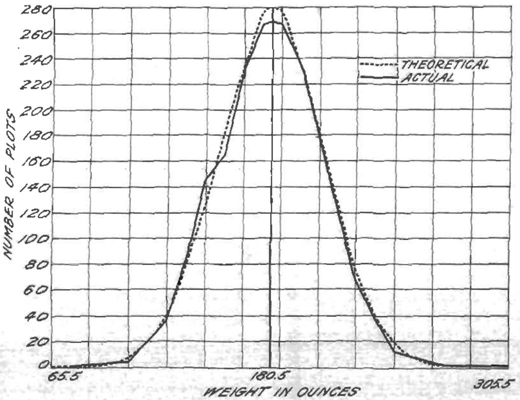

FIG. 3.—Frequency distribution of ultimate plot yields compared with normal curve of error

show probable border effect, but the third rows, while above the mean, differ from the mean only a little more than the probable error of a single two-row plot. The outside rod rows at the ends of each row (tiers 1 and 20) were not discarded, since their mean was only 1.95 per cent above the mean of the inside 18 tiers of rod rows.

Ultimate plots vary in yield from 75 to 302 ounces and fluctuate about a mean of a little over 180. The distribution of these plot yields is approximately normal, as may be seen from a comparison of the actual curve and the normal curve of error[3] in Figure 3. If a grouping in the tails of the curve is used, the probability[4] that devi-

[3] FELDMAN, W. M. BIOMATHEMATICS, BEING THE PRINCIPLES OF MATHEMATICS FOR STUDENTS OF BIOLOGICAL SCIENCE. p. 366-370. London, Charles Griffin and Company, Ltd. 1923.
[4] ELDERTON, W. P. TABLES FOR TESTING THE GOODNESS OF FIT OF THEORY TO OBSERVATION. Biometrika 1: [155]-163. 1902.

ations as great as or greater than those observed will occur in random samplings is 0.66, or, in two cases out of three, random samples would diverge as widely as the observed from the normal curve of error.

The agreement of actual and theoretical is very close and justifies determination of the normal curve constants for the ultimate plots. It does not follow that the curves of combination plots will approach the normal as closely as did that of the ultimate plots, but it may be assumed that the combination plots are fluctations of a normal curve and that they would take the expected form if the number were large enough.

Variation is here expressed in probable error as a percentage of the mean. The probable error of a single ultimate or 1/800-acre plot is 10.607 per cent of the mean.

$$y=\frac{N}{\sigma\sqrt{2\pi}}e^{-\frac{x^2}{2\sigma^2}}$$

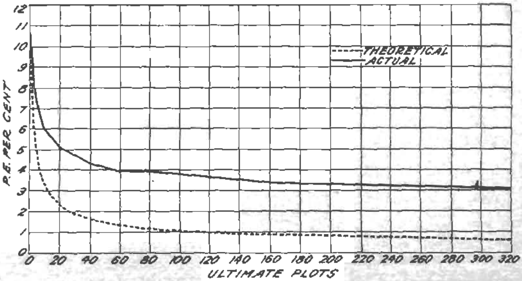

FIG. 4—Reduction in probable error due to increase in size of the plot

REDUCTION IN PROBABLE ERROR DUE TO INCREASE IN SIZE OF PLOT

The reduction in probable error due to increasing the size of the plot by grouping adjacent plots is given in Table 2 and Figure 4. Expected reduction by grouping like numbers of ultimate plots drawn at random also is shown. So far as possible in a series of increasing size, either the length or the width of the plots was kept constant and the area enlarged by addition in the other dimension.

Within the same general shape there is without exception a reduction in error due to the increase in the size of the plot. Of this method of comparing sizes Stadler[6] says:

> Increasing size of plot can hardly fail to decrease variability, for when two plots are combined the relative deviation can not be increased and is almost certain to be decreased, at least occasionally, by the canceling of plus and minus deviation. . . . But when increasing size of plot increases the size of the field, as it must if we retain the same number of plots, additional soil variations are almost always brought in, and variability of the larger plots is increased. In com-

[6] STADLER, L. J. EXPERIMENTAL ERROR IN FIELD-PLOT TRIALS. Paper read before Internatl. Cong.

paring large and small plots on the basis of equal numbers the advantage of large plots is therefore likely to be less than that indicated by the blank experiments.

TABLE 2.—*Reduction in probable error due to increase in size of the plot*

Approximate size of plot (acre)	Shape of plot			Probable error of single plot	Probable error expected with random selection of ultimate plots	Approximate size of plot (acre)	Shape of plot			Probable error of single plot	Probable error expected with random selection of ultimate plots
	Length	Width					Length	Width			
	Rods	*Rows*	*Feet*	*Per cent*			*Rods*	*Rows*	*Feet*	*Per cent*	
1/800	1	1	3⅓	10.607	10.607	1/400	2	1	3⅓	8.837	7.501
1/400	2	1	3⅓	8.837	7.501	1/200	2	2	6⅔	7.277	5.304
3/800	3	1	3⅓	8.083	6.124	1/100	2	4	13⅓	6.460	3.751
1/200	4	1	3⅓	7.686	5.304	1/50	2	8	26⅔	5.806	2.652
1/160	5	1	3⅓	7.087	4.744	1/25	2	16	53⅓	5.164	1.875
3/400	6	1	3⅓	7.053	4.331	2/25	2	32	106⅔	4.187	1.326
1/80	10	1	3⅓	5.921	3.355						
1/40	20	1	3⅓	5.100	2.372	1/200	4	1	3⅓	7.686	5.304
1/20	20	2	6⅔	4.312	1.677	1/100	4	2	6⅔	6.509	3.751
3/40	20	3	10	3.940	1.369	1/50	4	4	13⅓	5.812	2.652
1/10	20	4	13⅓	3.914	1.186	1/25	4	8	26⅔	5.314	1.875
1/5	20	8	26⅔	3.462	.839	2/25	4	16	53⅓	4.816	1.326
2/5	20	16	53⅓	3.024	.593	4/25	4	32	106⅔	3.865	.938
1/800	1	1	3⅓	10.607	10.607	1/160	5	1	3⅓	7.087	4.744
1/400	1	2	6⅔	8.479	7.501	1/80	5	2	6⅔	5.915	3.355
3/800	1	3	10	7.731	6.124	1/40	5	4	13⅓	5.244	2.372
1/200	1	4	13⅓	7.248	5.304	1/20	5	8	26⅔	4.793	1.677
1/160	1	5	16⅔	7.116	4.744	1/10	5	16	53⅓	4.280	1.186
3/400	1	6	20	6.720	4.331	1/5	5	32	106⅔	3.337	.839
1/100	1	8	26⅔	6.427	3.751	2/5	10	32	106⅔	2.373	.593
1/80	1	10	33⅓	6.378	3.355						
1/50	1	16	53⅓	5.552	2.652	1/80	10	1	3⅓	5.921	3.355
1/25	1	32	106⅔	4.458	1.875	1/40	10	2	6⅔	4.913	2.372
2/25	2	32	106⅔	4.187	1.326	1/20	10	4	13⅓	4.338	1.677
4/25	4	32	106⅔	3.865	.938	1/10	10	8	26⅔	4.094	1.186
1/5	5	32	106⅔	3.337	.839	1/5	10	16	53⅓	3.524	.839
2/5	10	32	106⅔	2.373	.593	2/5	10	32	106⅔	2.373	.593

In a comparatively uniform field large plots are likely to be more reliable than small ones, notwithstanding that the advantage generally may be less than that indicated by the results of the usual blank experiments. Table 3 shows a comparison of probable errors when they are derived from a constant number of plots of increasing size and from all plots of the field of corresponding sizes. The error of a 1/800-acre plot when the first 48 ultimate plots are considered is 9.771 per cent, while it is 10.607 per cent when the entire block is used. The errors decrease in about the same proportion until a 1/20-acre plot is reached, when they are the same since the whole block is used in each case. In this instance, increasing the plot from 1/800 acre to 1/20 acre with the total area concerned the same reduces the probable error about 60 per cent, whereas a like increase in the size of the plot and a proportionate increase in its area reduces it 56 per cent.

On the assumption that the probable error derived from the first 48 ultimate plots might be excessively high, it was calculated for a like number at each ¼, ½, and ¾ of the distance across the block and also starting from the other side. The probable errors are shown at the bottom of Table 3. In two cases they are approximately the same as for the first trial; in one the error is actually a little higher than that found from all plots in the field, and in the other it is considerably lower.

The deviation of the actual curve of reduction in error from the expected (fig. 4) is similar to that usually found in blank experiments and has been explained in several papers.

TABLE 3.—*Reduction in probable error due to increase in size of plot with the number of plots constant compared with reduction when using the entire block*

Size of plot (acre)	Number of plots constant			Using entire block		
	Number of plots		Probable error (per cent of mean)	Number of plots		Probable error (per cent of mean)
	Ultimate	Combination		Combination	Ultimate	
1/800	48	(48)	[a] 9.771	1,920	1,920	10.607
1/400	96	48	8.172	960	1,920	8.837
1/200	192	48	6.678	480	1,920	7.686
1/80	480	48	5.305	192	1,920	5.921
1/40	960	48	5.051	96	1,920	5.100
1/20	1,920	48	4.312	48	1,920	4.312
1/800	48	(48)	[b] 10.656 [c] 9.795 [d] 7.217 [e] 9.881	1,920	1,920	10.607

[a] Starting with 3d row of Table 1.
[b] Starting with 26th row of Table 1.
[c] Starting with 51st row of Table 1.
[d] Starting with 74th row of Table 1.
[e] Last 48 rod rows ending with row 98.

SHAPE OF PLOTS

Plots of the same size but of different shape are compared in Table 4. There is no decided or consistent advantage of one shape over another.

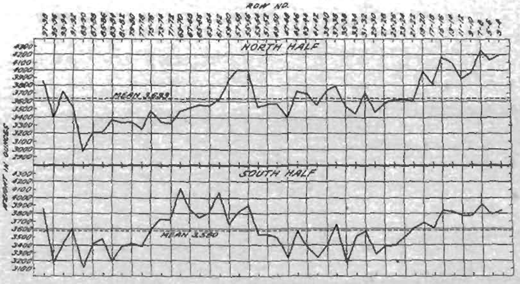

FIG. 5.—Variation in yield of 1/40-acre plots (10 rods by 2 rows) from the east side of the block to the west

This result may be accounted for in an examination of Figures 5, 6, and 7. Figure 5 is a graphic representation of yields of 1/40-acre plots (10 rods by 2 rows) from the east side of the field to the west; fluctuations in each are shown for the north half and the south half of the block. Figure 6 gives the variation from north to south; the block is divided into an east, middle, and west third, and the varia-

$\frac{1}{25}$ acre. Figure 7 shows a division of the block into high, medium, and low areas based on the yields of $\frac{1}{200}$-acre plots (1 rod by 4 rows).

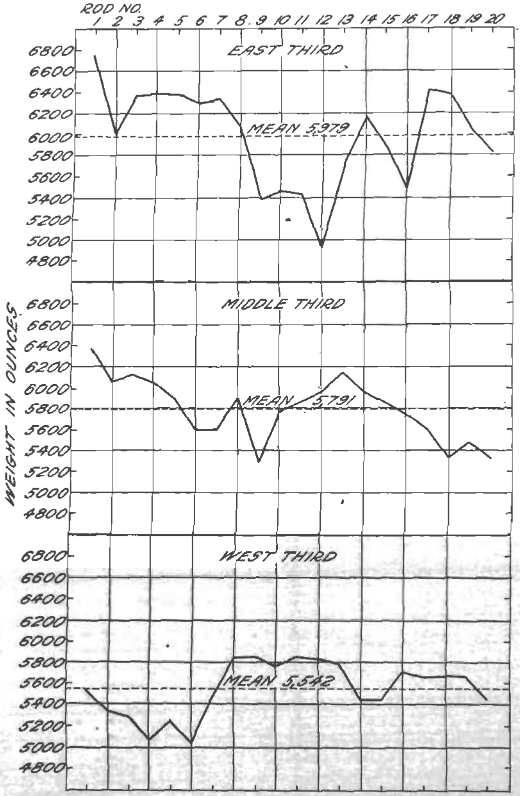

FIG. 6.—Variation in yield of $\frac{1}{25}$-acre plots (1 rod by 32 rows) from the north side of the block to the south

This grouping is arbitrary, but it conforms rather closely with Figures 5 and 6 and may be taken as roughly correct.

There is some general decrease in yield from north to south and

tion than in the other, and so it would not be expected that there could be much difference in the probable error, regardless of the direction in which the length of plots was extended.

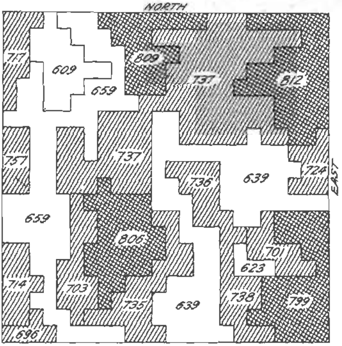

FIG. 7.—High, medium, and low yielding areas based on average yields of 1/200-acre plots (1 rod by 4 rows)

The outlines and the differences in means of the various groups in Figure 7 show that variation in this field was characteristically "spotted" rather than progressive from one side of the field to the other.

TABLE 4.—*Probable errors compared when plots are alike in size but different in shape*

Approximate size of plot (acre)	Shape of plot: Length	Shape of plot: Width		Probable error of single plot (per cent mean)
	Rods	*Rows*	*Feet*	
1/400	2	1	3⅓	8.837
	1	2	6⅔	8.479
3/800	3	1	3⅓	8.083
	1	3	10	7.731
1/200	4	1	3⅓	7.686
	2	2	6⅔	7.277
	1	4	13⅓	7.248
1/160	5	1	3⅓	7.087
	1	5	16⅔	7.116
3/400	6	1	3⅓	7.053
	1	6	20	6.720
1/80	10	1	3⅓	5.921
	5	2	6⅔	5.915
	1	10	33⅓	6.378
1/50	4	4	13⅓	5.812
	2	8	26⅔	5.806
	1	16	53⅓	5.552

Approximate size of plot (acre)	Shape of plot: Length	Shape of plot: Width		Probable error of single plot (per cent mean)
	Rods	*Rows*	*Feet*	
1/40	20	1	3⅓	5.100
	10	2	6⅔	4.913
	5	4	13⅓	5.244
1/25	4	8	26⅔	5.314
	2	16	53⅓	5.164
	1	32	106⅔	4.458
1/20	20	2	6⅔	4.312
	10	4	13⅓	4.338
	5	8	26⅔	4.793
1/10	20	4	13⅓	3.914
	10	8	26⅔	4.094
	5	16	53⅓	4.280
1/5	20	8	26⅔	3.462
	10	16	53⅓	3.524
	5	32	106⅔	3.337
2/5	20	16	53⅓	3.024
	10	32	106⅔	2.373

REPLICATION

Replication has been recommended and urged until rather few agronomy experiments involving but single plots are now in progress. The single-plot experiments of to-day are largely long-time tests that were established before so much emphasis was placed on replication.

The value and some of the difficulties of distributing plots have been considered in numerous papers, and part of the evidence has been derived from blank experiments. Generally a systematic method of distribution is used, because of the inconvenience in planting and harvesting with a random distribution. While not without exception, the curve of reduction in error by increased systematic replication usually approximates the curve expected from an increase in the number of plots with random distribution. If it does not, the system groups either similar or dissimilar yielding plots more often than would be expected from a random scattering. With the comparatively small numbers involved in field tests, even random distributions would result at times in deviations of considerable extent from those theoretically expected. However, the actual random distributions which have been reported have approached the expected more closely than the usual systematic replication.

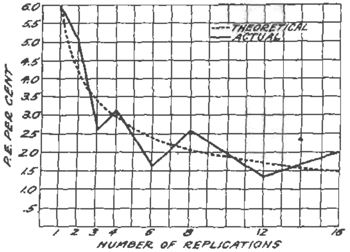

FIG. 8.—Reduction in probable error due to increased number of 1/80-acre plots systematically distributed and expected reduction due to increased number of plots distributed at random

Table 5 gives the probable errors of 1/80-acre and 1/40-acre single plots and of several replications[6] of each size up to 16; the expected errors with random distribution are added. Figures 8 and 9 show the same results. With each size the plots are 10 rods long, and since the block is 20 rods long there are two series across the field. The system of distribution made the units of a group in the second series fall halfway between the units in the first series.

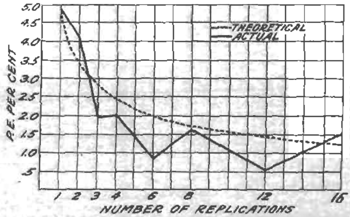

FIG. 9.—Reduction in probable error due to increased number of 1/40-acre plots systematically distributed and expected reduction due to increased number of plots distributed at random

Table 5 shows that the probable error has been reduced by replication. The reduction is not regular, however, and deviates at times rather widely from the theoretical. While sometimes above, the

[6] Number of replications is here used as the number of distributed plots and not the number plus 1.

deviations are more often below the expected. This means that plots yielding dissimilarly were grouped by the system of replication more often than would be expected from random distribution. Such results indicate that the probable errors of systematically distributed plots often may be too low. There is no justification for concluding that three replications are more reliable than four simply because in this particular trial a lower probable error was obtained with three than with four. The system of replication used is one of many that could have been tried, and each would have given somewhat different results. The general tendency is for lower error with increased replication.

TABLE 5.—*Reduction in probable error due to replication*

Number of replications	Probable error (percentage of mean) in—				
	1/80-acre plots (10 rods by 1 row)		1/40-acre plots (10 rods by 2 rows)		
				With systematic distribution	
	Expected with random distribution	With systematic distribution	Expected with random distribution	All of 96 plots	Number of groups constant at 16
Single	----------	5.921	----------	4.913	4.619
2	4.187	5.042	3.475	4.175	2.388
3	3.419	2.605	2.837	1.959	1.532
4	2.961	3.145	2.457	2.017	1.134
6	2.418	1.627	2.006	.844	.844
8	2.094	2.596	1.737	1.649	----------
12	1.709	1.319	1.418	.540	----------
16	1.480	2.022	1.228	1.522	----------

The same criticism that has been made with respect to the evidence obtained from blank experiments favoring increased size of plot (see p. 638) may be made of this evidence in favor of plot replication, namely, that in practice the area of ground used must increase in proportion to the number of replications, and thus additional soil variations are included. The last column of Table 5 shows the probable errors of 1/40-acre plots with the number of groups constant at 16 and the area of land increasing in proportion to the number of replications. The reduction in this case is almost as great as when the errors are calculated from the entire block. Other trials starting from various points in the field would give somewhat different results, but probably they would show reduction even though less than would be found when using the entire block.

VALUE OF REPLICATION IN UTILIZING A GIVEN AREA FOR EACH VARIETY

In the previous tables the number of varieties or the area of land has changed with plots of different sizes or with numbers of replicated plots. If only a limited acreage is available and a specific number of units must be tested, it is desirable to know the best utilization which can be made of this area. Assuming 1/10 acre devoted to each unit of a test, Table 6 gives probable errors of single 1/10-

1/10 acre. The smaller plots were distributed in the same sequence in each replication. The 1/20-acre and 1/40-acre plots were the full length of the block, but the 1/80-acre plots were only 10 rods long, which allowed two series across the block, and the system of distribution made the units of a group in the second series fall halfway between the units in the first series. The 1/160-acre plots were 5 rods long, which allowed four series across the block. The distribution was arranged so that the units of a group in the second, third, and fourth series fell one-half, one-fourth, and three-fourths, respectively, of the distance between the units of a group in the first series.

The probable error is very little lower with 16 replications of 1/160-acre plots than with 4 replications of 1/40-acre plots. The reduction in the probable error of single 1/10-acre plots amounts to 33 per cent for 16 replications of 1/160-acre plots and 31 per cent for 4 replications of 1/40-acre plots.

Table 6.—*Reduction in probable error due to replication within a given area*

Number of replications	Area of single plot (acre)	Shape		Probable error (per cent of mean)
		Length	Width	
		Rods	*Rows*	
Single	1/10	20	4	3.914
2	1/20	20	2	3.725
4	1/40	20	1	2.710
8	1/80	10	1	2.596
16	1/160	5	1	2.626

DISCUSSION

Very often the uniform land provided for experimental purposes near colleges and universities is rather limited in proportion to the number of men who desire to conduct independent investigations. Under such conditions it may be desirable to reduce the area of land devoted to any one test as much as possible and refine the methods.

On branch stations and substations the situation frequently is different. There may be plenty of comparatively uniform land, since the location is not influenced by convenience for a college staff, but often there is only one trained man to direct all of the work. It is to his advantage to conduct the field tests in such a manner as will require the minimum of supervision commensurate with a reasonable degree of accuracy.

One means of reducing technical labor and supervision is to arrange the routine tests so that they may be handled with ordinary farm machinery with which the more or less transient laborers are familiar. It then becomes desirable to use reasonably large plots rather than small plots with a large number of replications, if the error can be kept within limits that will give significance to varietal and cultural differences.

An additional advantage of conducting tests with standard farm equipment is that there is less likelihood of error in the application of results.

General observations in an experiment of this nature are fraught with some danger. The results, however, should give an indication of the limits within which the size of plot and the number of replications

should be confined. It is not to be assumed that probable errors for plots of the same size would be of like values with another crop, with the same crop in another year, or with the same crop on another block at the same station. Even a different system of replication with the same data would give somewhat different errors. Since the curve of actual reduction of error with increased replication is more often below the curve of expected reduction than above it, it would seem well to keep in mind the expected error.

In Table 2 the percentage of probable error of a single 1/40-acre plot is 3.914. Three times the error of a difference between two such plots is 16.6 per cent. Considering the error of three replications of 1/40-acre plots as 2.837 per cent, the expected error (three times the error of a difference between two replicated series) is 12 per cent, instead of 1.959 which was actually found (Table 5). Likewise for four replications of 1/40-acre plots and six replications of 1/80-acre plots, it is approximately 10 per cent. Additional replications necessary to reduce a significant difference below 10 or 12 per cent would hardly seem justified in sorghum-variety tests. Similar-yielding varieties often have other characteristics, such as resistance to lodging, fineness of stem, yield of grain in forage sorghums and forage in grain sorghums, and many other differences that influence the value of a variety even more than would a 10 per cent difference in yield.

Furthermore, it is usually assumed that a test should be continued for several years to include a sample of seasons. The magnitude of error from seasonal variation has been given little attention, except in a general way, but Engledow and Yule[7] and Stadler[8] have shown that it is rather large. They also have pointed out the futility of elaborate systems intended to reduce plot error to the minimum when seasonal error is not even measured.

SUMMARY

Green weights at maturity of the crop were taken on 2,000 rod rows of a selected uniform strain of feterita. The distribution of weights of 1,920 of these rod-row plots (borders excluded) formed a frequency polygon of the approximate shape of a normal curve. Plot variation as measured by the probable error of a single plot in percentage of the mean was determined for the rod-row plots and for larger plots consisting of various combinations of rod rows.

The probable error of a single rod row or 1/800-acre plot was 10.607 per cent, but this error was reduced consistently by taking successively larger plots of the same general shape. The error of a 2/5-acre plot was 3.204 per cent.

Owing to some decrease in yield both from north to south and from east to west and to the spotted tendency of high-yielding and low-yielding areas, no particular advantage of one shape of plot over another was found.

Systematic replication was effective in reducing error, but the reduction was irregular. Very often the error found was considerably below that which would be expected from a random distribution of the same number of plots.

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[7] Engledow, F. L., and Yule, G. U. THE PRINCIPLES AND PRACTICE OF YIELD TRIALS. Empire Cotton Growing Rev. 3: 112-146, 235-267, illus. 1926.
[8] Stadler, L. J. Op. cit.

In the trials made, the error was reduced nearly as much when the area of land was increased in proportion to the size of the unit plot or in proportion to the number of replications as it was when determined from the whole block.

Results of the test indicated that three or four replications of 1/40-acre or 1/80-acre plots will give results sufficiently reliable for the ordinary sorghum test.

SOME CHEMICAL AND MORPHOLOGICAL PHENOMENA ATTENDING INFECTION OF THE WHEAT PLANT BY OPHIOBOLUS GRAMINIS [1]

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INTRODUCTION

Take-all of wheat, caused by *Ophiobolus graminis* Sacc., was first discovered in the United States in Virginia in 1919. Since that time it has been found in most of the winter-wheat areas of this country. During the last few years some work has been, done on the disease and the causal fungus by Davis (*2*),[2] Kirby (*3*), McKinney and Davis (*4*), Webb and Fellows (*11*), and others in the United States. In Australia considerable attention has been given to take-all, and in France some work has been done on what is thought to be the same disease. However, Davis (*2*) is the only worker who has studied the microscopical and macroscopical phases of infection. The nature of Davis's work is briefly described in his summary as follows:

> Histological studies were made from plants exhibiting various stages of infection and these showed that the parasite entered the unbroken epidermis of the underground portions of the leaf sheaths, culms, and roots. The parasite first destroys the cortex of the roots and later enters the central cylinder. It also destroys the leaf-sheath and culm tissues and later enters the vessels, but it does not appear to make much progress after it enters the vessels.

The work on infection described in the present paper has been of two kinds, (1) morphological and histological and (2) microchemical. The purpose of the histological study was to learn what tissues were invaded and what morphological changes occurred in these tissues. The composition of the cell walls before and after infection was determined by microchemical methods. The roots, subcoronal internode, and crown were used in the histological study, but only the roots were employed in the microchemical studies. The results of the work described in this paper already have been a valuable aid in interpreting the behavior of wheat plants affected with take-all.

MATERIALS AND METHODS

Most of the histological studies were made with material embedded in paraffin. A combination of safranin and light green was found to be the best stain for differentiating the host and the parasite. This method of staining was especially satisfactory in lignified tissues where the host cell walls stained red and the fungus green.

Phloroglucin, used according to the method given below, was the best reagent for the detection of the fungus in the cell wall itself. Thin sections of fresh tissue were first treated 10 minutes in hot

[1] Received for publication Aug. 24, 1928; issued December, 1928. These investigations were conducted in cooperation with the Kansas Agricultural Experiment Station, Manhattan, Kans. Paper No. 238 of the Department of Botany and Plant Pathology.

[2] Reference is made by number (italic) to "Literature cited," p. 661.

hydrogen peroxide with frequent changes. The sections were then transferred to a 1 per cent solution of alcoholic phloroglucin on a glass slide and allowed to remain until the solution had mostly evaporated. One or two drops of concentrated hydrochloric acid then were applied, the cover slip placed in position, and the sections observed. Lignified cell walls showed red after this treatment, whereas the fungus hyphae passing through the walls showed as dark lines.

Kanred wheat was used in all of the studies described in this paper.

Where constant temperatures were maintained, soil-temperature control tanks, of the type developed in Wisconsin, were used.

Microchemical tests were made according to mimeographed directions received from Sophia H. Eckerson of the Boyce Thompson Institute for Plant Research (Inc.), Yonkers, N. Y. Other supplementary references were used (*1*, *9*). Briefly, some of the principal microchemical tests employed were as follows:

CELLULOSE: (1) Stain.—Iodine-potassium iodide+75 per cent sulphuric acid. (2) Solubility.—Copper oxide ammonia, 50 per cent chromic acid, zinc chloride and hydrochloric acid about 1:2.

HEMICELLULOSE: (1) Stain.—Furfural reaction with 1 per cent phloroglucin in alcohol and concentrated hydrochloric acid+heat; 4 per cent orcin and concentrated hydrochloric acid+heat. (2) Solubility.—Hot 3 per cent sulphuric acid.

CALLOSE: (1) Stain.—Resorcin blue (1:1,000), aniline blue dilute solution. (2) Solubility.—Cold 1 per cent sodium hydroxide or potassium hydroxide.

PECTIC SUBSTANCES: (1) Stain.—Ruthenium red (1:10,000 in water); methylene blue (1:1,000 in water). (2) Solubility.—Two per cent hydrochloric acid changes pectose to pectin or pectic acid; 2 per cent ammonia dissolves pectic acid.

LIGNIN: (1) Stain.—One per cent phloruglucin in alcohol+25 per cent hydrochloric acid; phenol-potassium chlorate mixture followed by concentrated hydrochloric acid. (2) Solubility.—Fifty per cent chromic acid. (3) Oxidation.—Hydrogen peroxide, potassium chlorate.

FURFURAL: (1) Stain.—Furfural reaction. (2) Removal.—Hydrocyanic acid.

SUBERIN: (1) Stain.—Sudan III. (2) Solubility.—Three per cent alcoholic potassium hydroxide. Insoluble in 50 per cent chromic acid.

MORPHOLOGICAL AND HISTOLOGICAL STUDIES

PRIMARY ROOTS

DESCRIPTION

The primary roots originate from the hypocotyl of the embryo and are the roots upon which the young plant depends for the intake of water and mineral nutrients before the crown and secondary or permanent roots arising from it are formed. The primary roots do not necessarily end their usefulness to the plant when the permanent roots are formed. Their duration as useful organs after this time varies according to conditions. Sometimes the primary roots may still be functioning when the plant is mature. On the other hand, many of the primary roots may die and disintegrate soon after the secondary roots are formed.

A cross section of a young primary root shows it to be composed generally of three parts—epidermis, cortex, and stele. (Fig. 1.) The epidermis consists of a single layer of elongated thin-walled cells. It is from these epidermal cells that the root hairs are developed and it is through the epidermal cells that infection occurs. The cortex lies just beneath the epidermis and is composed of four or five layers of large, thin-walled cells. The endodermis, the innermost layer of

the cortex, consists of a single, continuous layer of closely fitting cells. The outer tangential walls of the endodermal cells are thin, while the radial and inner tangential walls are thickened.

The pericycle, the outermost layer of the stele, consists of a single layer of radially elongated cells. The walls of the pericycle cells are slightly thickened. All the cells are nearly equal in size, except those opposite the xylem, which are smaller.

The xylem strands are seven or eight in number and alternate with the phloem. The protoxylem vessels are strengthened with spiral thickenings, but those formed later toward the center are pitted. The phloem bundles consist of series of three thin-walled cells. The conjunctive tissue of the stele consists of thin-walled irregular parenchyma cells. In the center of the stele is found a large central vessel with a heavy wall.

INFECTION

It is well to mention here that the hyphae of *Ophiobolus graminis* are of two kinds, distinguished by color and size. The macrohyphae are large in diameter, thick walled, and dark in color. The microhyphae are more slender, thinner walled, and colorless. The macrohyphae are ordinarily, though not always, found on the outer surface of tissues, and the microhyphae are generally found within the cells.

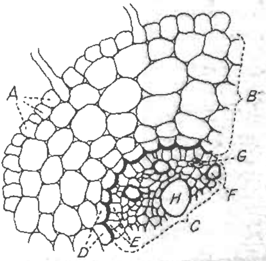


FIG. 1.—Sector of a cross section of a healthy primary root of a wheat plant. A, Epidermis; B, cortex; C, stele; D, endodermis; E, pericycle; F, xylem; G, phloem consisting of groups of three cells; H, central cavity. × 231

The macrohyphae, as they are ordinarily found on the roots, grow in contact with the epidermal cells. Where the macrohyphae are thus in contact with the epidermal cells, microhyphae may branch off at any point and penetrate the epidermal cells of the roots. The original penetration from the outside may or may not be attended with constrictions of the hyphae at the point of entrance. Once inside the root, the hyphae seldom undergo a change in size when passing through a cell wall except in instances where the cell wall undergoes a change in the immediate presence of such hyphae. These changes will be described later in this paper.

After entering the cortex the hyphae grow intracellularly from cell to cell in a radial or obliquely radial direction. In the cells of the cortex they have never been observed to parallel the long axis of the root. While the endodermis offers some resistance to the radial growth of the fungus, various segments of it differ in this respect. Certain portions may offer no resistance and others much. Finally the hyphae enter the stele and may penetrate any of its cells. A small lesion on one side of a root is sufficient to permit all of the vital conducting regions of the stele to become infected. The fungus that has entered one side of a root and has penetrated into the stele has never

been observed to pass out of the stele into the cortex on the side opposite the primary infection.

After entry into the stele the hyphae show a tendency to grow lengthwise of the root. This occurs especially in the xylem tubes or in the spaces created by the disintegration of the parenchyma and phloem of the stele.

It is well to mention here that a short time after complete or incomplete occupancy of the root the hyphae die and disintegrate completely. Therefore, the only indications of the earlier presence of the fungus are the malformations on the remaining cell walls or the destruction of cell contents and cell walls. The stele may or may not have been entered before this disintegration occurred.

The foregoing description may give the impression that the advance of the fungus through the invaded tissue is always fairly regular, but such is not invariably the case. In some cells the hyphae become aggregated much more than in others. The wall of a cell thus filled with mycelium becomes greatly thickened, making it difficult for the hyphae to pass from it to the next cell. In other cases the progress of a single hypha is in nearly a straight line so that it may be traced through four to five cells in succession. These variations in the behavior of the fungus may be due either to differences in food supply in the different cells or to mechanical or other differences in the nature of the cell walls themselves. In the former case the hyphae may remain and branch abundantly in the cells where the food is abundant, while in the latter case the hyphae may find it difficult to escape from some cells and thus be forced to remain. Aggregations of hyphae occur not only in single cells but also in groups of adjacent cells. Judging from the variations in behavior of the fungus in different cells, it seems that cells of the same tissue differ individually.

SECONDARY ROOTS

Description

The secondary roots, which form the bulk of the root system, are formed at or near the crown of the plant. Their number is not limited to a few (usually three), as is true of the primary roots. The crown is located at or somewhat below the surface of the ground and is the region in which several nodes are closely crowded together and from which tillers and leaves as well as secondary roots are formed. Secondary roots are formed not only in the original culm of the plant, but also grow from basal nodes of the tiller culms. New secondary roots may be forming for a considerable time during the early growth of the wheat plant.

The anatomy of the secondary roots does not differ greatly from that of the primary, except that their diameters are greater because of the greater quantity of parenchymatous tissue in the cortex and more abundant conjunctive tissue in the stele. As a secondary root becomes older, the walls of the outermost two or three layers of cortical cells become thickened. This thickening occurs first at the base of the root, that is, in the older portion, and gradually progresses toward the tip.

Infection

Infection and progressive penetration of the secondary roots by the fungus do not differ materially from those of the primary roots, with one exception. Within the thick-walled cortical cells, mentioned above, and in some of the cortical cells immediately outside of these, the hyphae become aggregated in large numbers, assume the form of macrohyphae, and grow lengthwise in the cells. These macrohyphae may become so numerous in a cell as to cause it to bulge and break. Furthermore, by means of microhyphae, the macrohyphae may penetrate outward through the cell wall, in which they are inclosed, in any direction rather than only toward the center of the root.

COLEOPTILE

Description

The coleoptile forms a complete sheath for the young growing plumule, its only opening being a slit near the apex. It is in reality a leaf folded longitudinally on itself with the two margins united. Within the cavity of the coleoptile is located the plumule.

In cross section the coleoptile is oval. At each extremity in the oval is a vascular bundle. The outer epidermis is composed of small cells more or less radially elongated and with their outer tangential walls slightly thickened. The cells of the inner epidermis, which are in more or less direct contact with the plumule, are similar to the cells of the outer epidermis except that they are elongated tangentially and the walls are not thickened. The tissue between the outer and inner epidermis is composed of large parenchyma cells with the exception of the two vascular bundles mentioned above. These parenchyma cells will be called in the present paper mesophyll. (Pl. 1, B.)

Infection

As the coleoptile becomes infected, macrohyphae are found closely appressed to the outer epidermis with their long axes parallel to the long axis of the coleoptile. These hyphae may be piled on one another many layers deep. Now and then a macrohypha ends off a side branch toward the epidermis. At the junction of the attacking hyphae and the attacked cell the hyphae may still be macrohyphae. As penetration of the host cell wall is effected a marked constriction occurs in the penetrating hypha. After emergence on the inner side of the wall the hypha may or may not enlarge somewhat.

Penetration continues through the mesophyll to the inner epidermis. When the inner epidermis of the coleoptile is reached by the hyphae they accumulate in great abundance in that vicinity and apparently are unable to proceed farther. Sometimes the inner epidermis may be infected, but more often it is not. The writer has never seen a case where the hyphae have penetrated the young leaves from the coleoptile, nor observed any lesions on the leaves as they emerge from the coleoptile. Seemingly the coleoptile acts as a protective organ for the inclosed young plumule during its early stages. A similar kind of protection has been noted by Reed and

Melchers (7) with respect to the attack of milo and feterita seedlings by *Sphacelotheca sorghi*.

It is true that the subcoronal internode, which often is infected, is surrounded by the coleoptile. However, the coleoptile has become ruptured by growth processes and often is mostly disintegrated and therefore can offer no further protection.

SUBCORONAL INTERNODE

Description

The subcoronal internode is the underground part of the stem of the wheat plant, below the crown. It is called a rhizome by Percival (5). On its upper end is the crown, and at its lower extremity are the primary roots and the remains of the seed.

A view in cross section shows the subcoronal internode to consist of essentially four parts, (1) epidermis, (2) cortex, (3) a cylinder of vascular bundles, and (4) the pith in the center. (Fig. 2.) The epidermal cells are closely appressed to one another and are consequently more or less square in cross section or elongated radially. The outer and radial walls are heavily lignified. The cortex has a thickness of six or seven layers of cells, the outer cells small, but gradually increasing in size toward the center. These cells are not especially thick walled. Occasionally there are groups of sclerenchyma cells in the cortex.

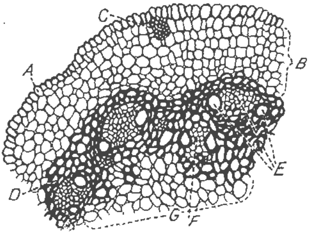


FIG. 2.—Portion of a cross section of the subcoronal internode of a healthy wheat plant. A, Epidermis; B, cortex; C, group of sclerenchyma cells; D, groups of thick-walled cells connecting with one another and making a complete cylinder surrounding the bundles and pith; E, large bundle; F, small bundle; G, pith. ×96

The bundles are arranged in a circle of seven large ones alternating somewhat irregularly with seven smaller ones. Each bundle is surrounded by thick-walled lignified cells, which are joined from bundle to bundle, thus forming a continuous irregular circle inclosing all the bundles and the pith. The pith is composed of large, thin-walled parenchyma cells loosely joined together.

Infection

Infection of the subcoronal internode is similar to that of the roots; that is, infection occurs through the epidermis, at any point. The hyphae proceed though the cortex and bundle sheaths into the pith. Any cell may become infected. The hyphae, after entering the xylem tubes and thick-walled cells of the bundle sheaths, assume the form of macrohyphae and grow lengthwise in these cells. This lengthwise growth of a single hypha has been traced as far as 1 cm. and doubtless may extend farther.

CROWN

The primary purpose of the present study of the crown was to learn the behavior of the crown and of the fungus in the crown of plants that were able to recover from the take-all disease. The crown is of particular interest from this standpoint because in such plants it has been only partially invaded. In a recovered plant the primary roots, the subcoronal internode, the first, second, and third leaves, and some of the secondary roots, expecially the lower ones, all may be destroyed, while the upper part of the crown and the higher secondary roots may be free from the disease. Such recovered plants are able to grow to maturity and produce a normal head. As pointed out by Davis (*2*), such upper secondary roots no doubt may escape infection on account of their later formation. The crowns of recovered plants are infected only in part. As soon as infection becomes general in the crown the entire plant dies and no recovery is possible.

Description

The crown is the region in which the tillers, leaves, and secondary roots are formed. (Fig. 3.) The elongation of the culm, however, carries some of the leaves up away from the crown. The crown is surrounded by the sheath of the first leaf and partially also by the sheaths of the other lower leaves.

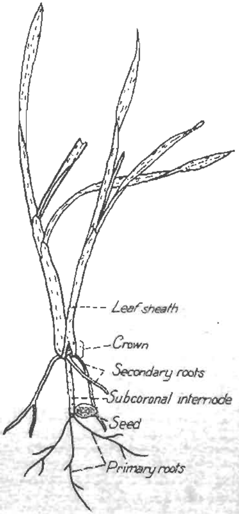


Fig. 3.—Diagram of a young wheat plant illustrating the location of the various parts. Subcoronal internodes may vary greatly in length

The lower true leaves and secondary roots, which latter usually come in pairs, alternate with each other vertically on the stem. The first true leaf comes at the junction of the subcoronal internode and the crown. The first two secondary roots come just above the attachment of the first leaf, one on each side at points about 90° from the middle of the base of the leaf. In growing outward each of these two secondary roots penetrates the base of the first leaf and thus ruptures it at two points. The second and other successive lower leaves alternate with the second and other pairs of secondary roots in a similar manner. Thus the leaves are two ranked on the stem, and the second and other successive pairs of secondary roots come approximately at right angles to each preceding pair.

The first, second, and third leaves receive their vascular traces directly from the bundles found in the subcoronal internode. The

trace of the fourth leaf, however, does not connect directly with the vascular strands found in the subcoronal internode, but with branches of these. These branches come nearly at a right angle and meet the traces of the fourth leaf near the center of the crown. The fourth leaf, however, does not depend entirely upon the branches of the bundles from the internode for its supply of water and nutrients, for it is also connected with the vascular systems of the second pair of secondary roots. Subsequent leaves are connected with the vascular systems of the second and third pairs of secondary roots by means of branches of bundles sent from these roots to near the center of the crown where they connect with the leaf traces.

As pointed out above, the first two secondary roots come from the crown just above the attachment of the first true leaf. Their vascular systems connect directly with the established system coming from the subcoronal internode. The vascular systems of the second pair of secondary roots also connect directly with the bundles from the subcoronal internode and also establish an independent vascular system extending up the culm. This independent system branches toward the center of the crown, connecting with the fourth and fifth leaf traces and extending into the apical bud.

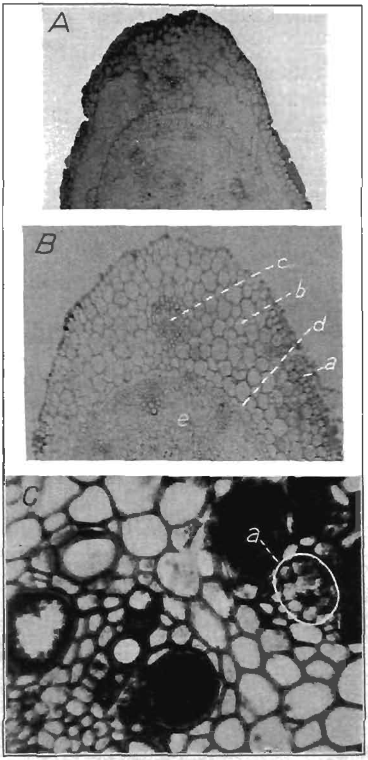
The vascular systems of the third pair of secondary roots have no connection with the bundles from the subcoronal internode but are independent and branch toward the center of the crown, connecting with leaf traces and extending to the apical region.

INFECTION

As noted above in discussing the infection of the subcoronal internode, the parenchyma cells of the cortex may become infected. There is an extension of these parenchyma cells into the crown for a short distance, but for some reason there is a sharp line of demarcation between the infected cells in the subcoronal internode and the uninfected cells in the crown. The parenchyma cells of the pith also extend into the crown. These cease in the region where the vessels from the subcoronal internode branch toward the center to connect with the traces of the fourth leaf. The latter parenchyma cells are infected as far as they extend into the crown but no farther.

If one cuts a longitudinal section in a median plane of the crown of a recovered plant, this pith region with its darkened infected cells may be seen to extend into the crown in a cone-shaped area. The apex of the cone is just below the branches of the bundles from the subcoronal internode to the traces of the fourth leaf.

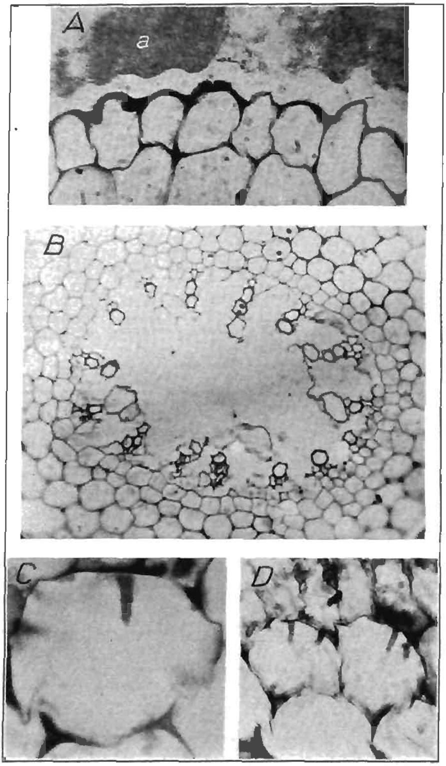
The hyphae in the cortical cells of the subcoronal internode extend out into the parenchyma cells of the first leaf. The hyphae growing lengthwise in the xylem cells of the subcoronal internode grow up into the xylem of the first leaf and to some extent may pass into the vascular strands of the first pair of secondary roots. It is noticeable that the hyphae become fewer and fewer in the xylem strands as the top of the subcoronal internode is approached. This diminution is more marked as they enter the crown. The same is true of the hyphae that enter the crown through the secondary roots. The reason for this will be discussed under a separate heading.



A.—Portion of a cross section of an infected coleoptile in which the mesophyll is disintegrated. The outer epidermis and most of the first layer of cells beneath it, the inner epidermis, the bundle, and the cells in its vicinity are not disintegrated. × 57

B.—Portion of a cross section of an infected coleoptile in which the cells of the mesophyll are not disintegrated: *a*, Outer epidermis; *b*, mesophyll; *c*, one of the two fibrovascular bundles; *d*, inner epidermis; *e*, leaves of the plumule. × 67

C.—Portion of infected vascular region of the subcoronal internode. The xylem tubes have become partly or completely clogged by products from their own walls; *a*, disintegrated phloem. × 500



A.—*Portion of outer epidermis of the coleoptile showing thickening of outer tangential walls of epiderma* cells caused by the incrusting layer of hyphae; the epidermal cells are not penetrated. The layer of hyphae *a* had been somewhat pulled away from the epidermis in the preparation of the section. × 330

B.—Stele of a primary root largely disintegrated by *Ophiobolus graminis*, with the xylem tubes and some of the parenchyma and pericycle cells in their immediate vicinity still intact; the walls of the endodermis have become thin. × 400

C and D.—Lignitubers in the cortical cells of roots of young wheat plants. × 1,000

MORPHOLOGICAL CHANGES IN CELL WALLS IN BASAL PORTIONS OF INFECTED PLANTS

CHANGES IN THE ROOTS

The morphological changes in the cell walls of the primary and secondary roots are very similar and will be discussed together. Changes that occur in the wall of one cell may be very different from those that occur in another, although the two cells may be comparatively close to each other, of a similar type, and have equal quantities of hyphae in them. Accordingly, the description of a change in a certain cell wall can not apply to the walls of all infected cells of that particular type.

Shortly after or even before the entry of mycelium into a cell its wall may become very much thickened. This thickening may or may not be uniform on all surfaces of the wall. It is especially noticeable at the corners. The thickening is not necessarily formed on the side of the wall toward the lumen, for it may form between the cells, or the intercellular spaces may be filled with it.

Somewhat similar cell-wall thickenings in diseased plant tissues have been observed by other investigators. Ravn (*6, p. 113*) described and figured cell-wall thickenings in oats attacked by *Helminthosporium avenae* Eidam. The composition of these thickenings was not definitely determined, although it was stated that they stained intensely with thionine and other basic stains.

Tisdale (*10*), working with flax wilt, pointed out that certain cell walls of the flax plant may become thickened in advance of the hyphae of *Fusarium lini* Bolley, and he attributed the thickening to the formation of suberin.

When a wheat root becomes infected with *Ophiobolus graminis* very noticeable changes occur in the walls of the infected cells in some cases. When an infecting hypha starts to penetrate a cell wall a slight protuberance is formed on the cell wall at a point just opposite. The protuberance elongates at right angles to the wall, becoming longer and longer in front of the hypha as the latter advances. (Pl. 2, C andD.) As the hypha progresses it becomes more and more attenuated. Finally either the hypha is able to outgrow the protuberance and enter the lumen of the cell or the protnberance is able to prevent its doing so. If the hypha passes through the protuberance, the hypha immediately increases in diameter to normal size again.

Protuberances in wheat cells caused by other organisms but similar to those described above have been observed by others, and the names callosities and calluses have been applied to them (*8, 12*). Either of these names would be a misnomer if applied to the abnormalities produced in wheat by *Ophiobolus graminis*, as microchemical tests have shown that the protuberances here described contain no trace of callose but rather are composed chiefly of lignin. Therefore the writer suggests the name "lignitubers" for the protuberances caused by *O. graminis*, the name alluding to their composition and form. This designation will be used in the present paper.

In form the large lignitubers have the general shape of a finger. (Fig. 4.) The surfaces are generally smooth and slightly undulating. The bases flare out and are joined to a similar substance on the inner surface of the cell walls. The same substance may occur also between the walls of the adjoining cells where the hypha penetrates. When the

lignituber is viewed sideways a lighter staining median line may be seen extending lengthwise through or almost through it. This line is the hypha contained within. When viewed in cross section a lignituber resembles a doughnut, the central hypha corresponding to the hole in the doughnut. In size these lignitubers may vary greatly. Some are very minute; others are sufficiently long to cross completely the cell lumen.

Lignitubers do not invariably accompany penetrating hyphae. They are usually found most abundantly on walls of cells that thicken their walls otherwise in the presence of the attacking organism, and they may occur in cells of all the different tissues of the root.

In part of the cortex of the proximal portion of the secondary roots, about 0.5 cm. from the crown, it was found that the hyphae after entering certain cells often grew lengthwise rather than always penetrating the cells crosswise—that is, in the radial direction—as was the usual procedure in portions of the roots farther from the crown. In seeking an explanation for this behavior of the hyphae in those particular cortical cells close to the crown the corresponding tissues of a healthy root were examined. It was found that there occurred in the cortex of the root, near the crown, at a depth of one or two layers of cells below the epidermis, a ring of cortical tissue with rather thick cell walls. This ring was three or four cells in thickness. It seemed that the ability of these cortical cells to thicken their walls might account for the rather peculiar behavior of the hyphae in this tissue. This might occur in two ways. Hyphae that were in these cells when the thickening was taking place found difficulty in escaping radially and as a result grew lengthwise in the cells; or these cells, because of their inherent ability to thicken, were able to produce thick walls quickly, due to the stimulation of the fungi within, and thus forced the hyphae to grow lengthwise in the cells.

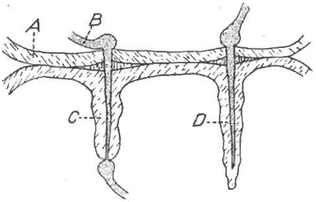


FIG. 4.—Diagram showing penetration of cell walls by infecting hyphae and lignitubers formed about them. A, Cell wall; B, hypha; C, lignituber through which the hypha has passed; D, lignituber through which the hypha has not passed. (See pl. 2, C and D.) × 3000

After the stele of either the primary or the secondary roots becomes invaded a disintegration of certain of the cells within may occur. When this happens the phloem and most of the parenchyma of the stele disintegrate, the phloem usually more rapidly than any of the other tissues. The walls of the xylem vessels, as well as the walls of the parenchyma and pericycle cells relatively close to the xylem, remain intact. (Pl. 2, B.) Such disintegration does not always occur, but it usually does. In cases of such disintegration the distal portion of the root beyond the lesion, of course, functions no longer.

Another notable change occurring in the bases of such infected secondary roots is the clogging of the xylem vessels and sometimes of the surrounding cells. The walls of the xylem vessels may become thickened to such an extent that the lumen often is entirely filled.

Changes in the Coleoptile

In a preceding paragraph it was mentioned that the macrohyphae accumulate in large numbers on the outer epidermis of the coleoptile. The presence of these hyphae, before they penetrate the cell walls of the epidermis, causes these walls to become greatly thickened with lignified material. (Pl. 2, A.) This thickening is most pronounced on the outer, tangential walls, but it also extends down the radial walls. When penetration of the epidermal wall occurs the hyphae apparently are always accompanied by lignitubers, many of which are larger than those seen in any other cells. The row of cortical cells immediately below the epidermis reacts similarly but not to such a marked degree. The walls of the other cells of the coleoptile mesophyll neither become thickened nor form lignitubers in the presence of the hyphae. The cell walls of the inner epidermis become slightly thickened but apparently do not form lignitubers.

Comparatively soon after infection the mesophyll cells of the coleoptile disintegrate completely with the exception of those near the two bundles. After this disintegration the two empty spaces thus formed appear as two crescents with their concave sides toward each other. The two bundles with the immediately surrounding mesophyll separate the two ends of these crescents. (Pl. 1, A.)

Changes in the Subcoronal Internode

As stated previously, in a healthy plant the outer tangential walls of the epidermal cells are thickened. Apparently no additional thickening occurs after penetration by the fungus. On the other hand, the cell walls of any or all of the cells of the cortex may become thickened after penetration. This thickening is especially noticeable in the intercellular spaces. These become completely filled with a substance staining strongly with safranin.

The greatest changes that were noticed after infection were in the xylem tubes and some of the thick-walled cells surrounding the bundles. It was found that the walls of these cells became thickened to such an extent that the lumen became entirely filled. Most of the xylem tubes became completely plugged. This plugging occurred in advance of the hyphae or when they were present. (Pl. 1, C, and fig. 5.) In the latter case it was noticeable that the hyphae became disintegrated. Undoubtedly such plugging hinders considerably the upward progress of the hyphae into the crown. In this way the plant may be helped to recover from the disease, provided secondary roots are formed in large enough numbers to provide for the needs of the young plant after the primary roots have been cut off by the plugging of the vessels leading through the subcoronal internode.

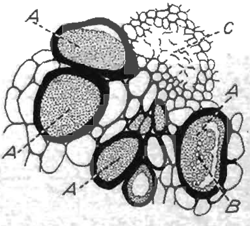


Fig. 5.—A diseased vascular bundle of the subcoronal internode. A, Xylem tubes that have become clogged or partly clogged with an outgrowth of their own walls, some of the smaller cells in the vicinity having also become clogged or partly clogged; B, a group of hyphae that are surrounded by the clogging material within a xylem tube; C, disintegrated cells of the phloem region. (See pl. 1, C.) × 400

As was the case in the roots, the phloem of the bundles in the subcoronal internode, as well as the pith, often disintegrates in the presence of the parasite.

CHANGES IN THE CROWN

As previously noted, studies of the crown have been made only on plants that have recovered from the disease. In such plants the hyphae have entered the crown through the xylem tubes of the subcoronal internode and lower secondary roots or through the pith of the subcoronal internode. The progress of the hyphae entering through the xylem tubes is so much hindered by the great thickening of the walls and subsequent plugging of these vessels that further progress in some tubes may be stopped. The hyphae entering through the pith disintegrate this tissue in the crown to a point just below where the vessels from the subcoronal internode branch off to the fourth leaf. The further progress of these hyphae is probably hindered by the rather thick, relatively resistant cell walls in the region of the branching.

MICROCHEMICAL STUDIES OF HEALTHY AND DISEASED ROOTS

As previously noted, infection of the basal portions of the wheat plant by *Ophiobolus graminis* is accompanied by certain morphological changes. Certain cell walls become thickened, and peculiar outgrowths called lignitubers are formed. It was the object of the microchemical studies, the results of which are here summarized, to determine what chemical changes, if any, occurred in the walls of the infected cells, (1) where morphological changes were evident and (2) where no morphological changes were noticeable.

The plants used in these studies were, on an average, 9 days old. They were grown in steamed soil subsequently infested with a pure culture of *Ophiobolus graminis* isolated from wheat in Kansas. The infested soil was placed in large test tubes, and disinfected seed of Kanred wheat was sown in it. These tubes were then kept at a temperature of about 20° C., and the moisture of the soil was kept at about 55 per cent of its moisture-holding capacity. The results are given in Table 1.

TABLE 1.—*Comparison of cell-wall constituents of healthy and diseased primary and secondary roots of young Kanred wheat plants grown in large test tubes at about 20° C.*

CELLULOSE

Cell-wall constituents in healthy roots	Cell-wall constituents in diseased roots
All of the cell walls, including those of the root hairs, had their foundations of cellulose.	In general some cellulose was present in all cell walls, but it varied more in diseased than in healthy roots. Many cell walls of the cortex had lost all or part of their cellulose. The endodermis and parenchyma of the diseased stele had less cellulose than the healthy material. The lignitubers had no cellulose so far as tests showed. The pericycle of the diseased plants had little cellulose in comparison to that of healthy plants.
HEMICELLULOSE	
The only form of hemicellulose present was pentosans, found in the xylem.	No change was produced by disease.
CALLOSE	
The test for callose was negative.	None was found. Evidently the disease did not cause its production.
PECTIC SUBSTANCES	
Pectose was the only pectic substance present. It was located in the root hairs and epidermal cell walls.	No change was produced by the infecting organism.
LIGNIN	
Lignification occurred only in the walls of the xylem tubes.	The walls of the cortical cells that were thickened in the presence of the organism showed large amounts of lignin and comparatively little cellulose. The lignitubers and the substance filling the intercellular spaces in the cortex were largely lignin; likewise some striations in the inner tangential walls of the endodermis showed lignin. The parenchyma of the pith and the pericycle near the xylem had become lignified.
SUBERIN	
Suberin was present in the xylem and radial walls of the endodermis. In the xylem it was located in the thickenings.	A slight amount of suberin had been produced in the thickenings of the infected walls of the cortex. There was also a slight quantity in the lignitubers. Neither the cell wall thickenings nor the lignitubers were completely soluble in chromic acid, but they were mostly so.

SUMMARY

The studies here reported pertain to infection of the wheat plant by *Ophiobolus graminis* Sacc., the cause of the disease known as take-all. It has been shown which of the tissues studied are invaded and what are the morphological and chemical changes produced by such invasion. The microchemical studies here reported were made on the roots only.

The behavior of the primary and secondary roots in the presence of the parasite is very similar. The fungus penetrates the epidermis, passes through the cortex, and enters the stele. Its progress is hindered to some extent by the endodermis.

Many of the cell walls with which the parasite comes in contact become thickened, or the walls may produce elongated protuberances in front of and around the invading hyphae. These previously undescribed structures are given the name lignitubers.

All the cells and vessels of the stele, except the xylem tubes and some cells near it, may be distintegrated after penetration.

Microchemical studies made on diseased roots showed that the disease caused a nonuniform reduction of cellulose, which was replaced by lignin and a slight amount of suberin. This is especially true of the thickened cell walls and the lignitubers. Other substances are not changed by the disease.

The coleoptile is penetrated through the unbroken epidermis, which is greatly thickened, and many lignitubers are formed in its cells. During the progress of the hyphae inward the mesophyll is destroyed except that near the bundles. The inner epidermis stops the further progress of the fungus and thus the coleoptile protects the young seedling, at least to some extent.

The subcoronal internode is attacked through the epidermis and may become infected in all parts. The pith and the phloem may become disintegrated. The xylem tubes and cells in their vicinity respond to the presence of the organism by thickenings on their inner walls by which they may be filled completely.

Studies of the crown were made on plants that had recovered from the disease. In these plants the lower part of the crown and the lower secondary roots were found to be injured. The hyphae had entered the crown through the pith and xylem tubes of the subcoronal internode and the xylem of the lower secondary roots. In many cases the xylem tubes where they enter the crown were plugged by excessive thickenings on their inner walls.

The results here reported have been found useful in interpreting the behavior of wheat plants affected with take-all and may have a bearing on the explanation of resistance, if resistant plants are found.

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THE INOCULATION OF PACIFIC NORTHWESTERN RIBES WITH CRONARTIUM RIBICOLA AND C. OCCIDENTALE[1]

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INTRODUCTION

The native piñon blister rust or nut-pine rust (*Cronartium occidentale* Hedgc., Beth., and Hunt) (*11*),[3] which has been known in Colorado for many years on species of Grossulariaceae, is now known to extend westward into southern California and northward into the inland regions along the Nevada-California boundary and into southern Idaho. One collection of this rust was made as far north as Spokane, Wash., on *Ribes aureum* Pursh, in 1914 (collection No. 6360 by W. E. Flowers, FP No. 41916[4]). Baxter (*1*) found the rust in northern Wyoming south of Buffalo. *C. occidentale*, although widely distributed, has little economic importance.

The white-pine blister rust (*Cronartium ribicola* Fisch.), which is believed to have been introduced into the Pacific Northwest on a single importation of eastern white pines from France (*2*), like the piñon rust, occurs on species of Grossulariaceae, but its alternate stage attacks 5-needled pines. This rust is now thoroughly established in British Columbia and has been found at numerous points in both eastern and western Washington (*19*)[5] and in Oregon. It is known to exist in one county in Idaho. Undoubtedly it will continue to spread eastward and southward. This rust has shown itself capable of causing great damage to *Pinus monticola* D. Don., the species on which forestry in northern Idaho mainly depends. The present encroachment upon the forest areas of this region by the white-pine rust is of the gravest concern to the future of forestry in Idaho and should be the immediate signal for localized control work.

Advance infections of both rusts are found in their uredinial stages on species of the Grossulariaceae, which for convenience in this paper will be usually grouped under the name Ribes. A method is therefore needed by which any Cronartium found on Ribes in Idaho can be definitely assigned to the proper species. When *Cronartium ribicola* begins to invade the range of *Pinus lambertiana* Dougl. in California, a similar need will be felt there. The rusts are morphologically quite distinct on pines (*6*), but their stages on Ribes can not be certainly distinguished by any morphological criteria so far developed. Hedgcock's observations in the field and in the green-

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[3] Reference is made by number (italic) to "Literature cited," p. 681.

[4] Specimen collection number of pathological specimens for study, Office of Forest Pathology.

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house show that the telial columns of *C. occidentale* (*11*) are somewhat darker colored (walnut brown to Vandyke brown (*20*)) than the Sanford's brown ones of *C. ribicola*, and the average length and the thickness of wall of its urediniospores are significantly greater (*5*). Some of the Cronartium specimens, however, are so near the border line separating the two species that their identification by any of these characters becomes extremely dubious.

Because of the situation just described, an investigation was undertaken to differentiate physiologically the uredinial stages of the two Cronartiums by studying in the greenhouse their reactions on various species of Ribes. With this end in view, an extensive collection of native and cultivated Grossulariaceae, representative of those found in different parts of the United States, Canada, and Europe, were propagated at the pathological greenhouses of the United States Department of Agriculture, Washington, D. C. When these plants became available for inoculation purposes they were inoculated with the two blister rusts under similar conditions. For these comparative inoculations environmental conditions were maintained as favorable as possible for rust infection and development. The results given in this paper are those obtained from the inoculation of Ribes hosts from the Pacific Northwest, which are potentially hosts of these two blister rusts.

VALUE OF GREENHOUSE INOCULATION DATA

Results with *Cronartium ribicola* on the Pacific Northwest Ribes are of especial value in indicating the possibilities of the spread of this introduced disease in that region. While greenhouse inoculation results do not always tell the same story as field tests, because of the uncertainty and extreme variability of field conditions, nevertheless the former are of particular value. As a general rule, when experimental field tests with rusts have been supplemented by greenhouse tests, it has been found that the artificial inoculation results obtained in the greenhouse indicate with considerable accuracy what may be expected to occur in the field when a given host species or variety is exposed to infection.

The importance of agreement between greenhouse and field conditions has been pointed out by other rust investigators. According to Spaulding (*21*), the estimates for the inside inoculation experiments with *C. ribicola* on Ribes species and varieties agree surprisingly with those made entirely independently out of doors. Fromme and Wingard (*10*), in their investigations of the varietal susceptibility of beans to rust, also give evidence that greenhouse tests that have supplemented field tests form a reliable index of the field behavior of a variety with respect to rust infection. Melchers and Parker (*15*) in a comparison of nursery and greenhouse results agree with them, for they report that their results with wheat rust in the greenhouse were similar to those produced under field conditions in the rust nursery where conditions were as severe as those to which a commercial field is subjected in a natural epidemic. While field tests must finally decide the susceptibility of any species or variety of Ribes to either piñon or white-pine blister rust, the greenhouse results give an excellent indication of what may be expected in the field under conditions favorable for infection.

When a Ribes species proves fully susceptible in the greenhouse under controlled conditions but produces a reduced number of urediniospores in the field, this difference can usually be explained either by field conditions unfavorable to infection or by the growth habits of the host; i. e., the time and manner of leaf production, or the distance from infected white pines. Disagreements would, therefore, be expected in comparing the order of relative susceptibilty of Ribes species in the greenhouse and in the field.

SOURCE OF THE INOCULUM

Cultures of the two Cronartiums were obtained from the field from representative localities. No attempt was made to procure single-spore lines. For the purposes of the experiments a mixture of lines from the same locality was not regarded as objectionable. As has been stated above, the problem was chiefly the differentiation of the two species of Cronartium on Grossulariaceae, which are heterozygous hosts. Evidences of geographic strain differences for each of the two species were, however, also observed.

Strains of *Cronartium ribicola* were obtained from New England and from British Columbia in the Pacific Northwest, and in 1922 a strain from Scotland was also secured. Strains of *C. occidentale* were obtained from Colorado, southern California, Nevada, and Wyoming.

BEHAVIOR OF THE BLISTER RUSTS IN GREENHOUSE INOCULATION

The best urediniospore infection was generally secured during the late summer and fall upon leaves that had fully expanded but had not commenced to harden. Stakman and Piemeisel (*23*) recorded *Puccinia graminis* as developing unusually well in late September and early October, a period that they found ideal for rust development in the greenhouse. Spaulding (*21*) likewise observed that leaves produced by buds developing in late summer or fall, even if very small, readily become infected. High temperatures in the late spring, summer, and early autumn interfered seriously with successful inoculations. At the beginning of cooler weather, during the autumn period, the cultures kept over the summer in the greenhouse revived vigorously upon congenial Ribes hosts.

Uredinia of both *Cronartium ribicola* and *C. occidentale* generally appeared within 8 to 14 days. A large number of comparative inoculation experiments conducted for the purpose of determining the incubation period of uredinia under the same inoculation and infection conditions showed no essential difference between the two species. The average length of time between the actual inoculation of Ribes and the production of mature urediniospores was 11 days. Uredinial development was most vigorous during the bright, sunny weather of spring and early fall; cloudy weather retarded rust development, appreciably delaying the appearance of uredinia, especially during the winter months. Under favorable conditions abundant stock cultures of strains of both rust species, systematically maintained for the purpose of supplying spores for inoculating purposes, could be readily procured within approximately the same period of time upon congenial species of the *Ribes aureum* group (*R. aureum*, *R. odoratum* Wendl., and *R. gracillimum* Cov. and Brit.) or upon certain varieties of *R. nigrum* L. Such stock cultures persisted in a good condition for approximately three weeks, when the most heavily

infected leaves began to dry up and drop from the host plant. A successful maintenance of vigorous stock-culture material was largely dependent upon attendant weather conditions. Artificial inoculations proved to be quite necessary in order to insure the continuous propagation of the different strains, because self-inoculation could not be depended upon. Even on heavily infected plants leaves that were uninfected rarely became infected as a result of self-inoculation.

A moderate temperature up to 75° F. (24° C.), the temperature at which Ribes plants make their best development in the greenhouse, was found to be most favorable for uredinial development of the two Cronartiums. This is coincident with the finding of Stakman and Piemeisel (*23*) for *Puccinia graminis*. Mains (*14*) states that a temperature of 16° to 20° C. is most favorable for the germination of rust spores, few germinating below 5° or above 30°. Peltier (*18*) gives 20° C. as the optimum temperature for infection and subsequent development of stem rust. According to Doran (*7*), the minimum, optimum, and maximum temperatures for the germination of urediniospores of *Cronartium ribicola* are 8°, 14°, and 25° C., respectively. During exceptionally warm weather in the greenhouse under conditions of high temperatures, congenial plants, ordinarily heavily infected, were observed to show only a very much reduced number of uredinia. Pustules that developed under such conditions might become aborted or otherwise abnormal.

To insure maximum rust infection an atmosphere close to saturation was necessary, and for normal development a considerable amount of sunlight was needed. Peltier (*18*) has observed that where other environmental factors are equal, the duration of the period of sunshine is more important than its intensity in influencing the production of rust infection.

The rusts infect unhardened leaves most readily and vigorously. Stakman and Piemeisel's best infection results with *Puccinia graminis* (*23*) were generally obtained on young leaves inoculated at an early stage of development, although in the case of some grasses the older leaves became infected more readily. Evidence that the older Ribes leaf is more susceptible to infection from *Cronartium occidentale* has also been obtained. The data will be presented in another paper.[6]

As a general rule matured senescent leaves produced fewer uredinia and favored the development and production of telia. Telia developed on Ribes when metabolism and growth were apparently slowed up. Mains (*14*) states that this same condition exists in the case of *Puccinia triticina*, which he has never observed to produce telia except on old leaves when the wheat plants approached maturity. Matured leaves of certain Ribes species bore neither uredinia nor telia and were quite immune to infection. In midwinter it was a common experience to secure on young leaves of the *Ribes aureum* group mostly uredinia of both rust species. For both Cronartiums a relationship is thereby indicated not only between the age of the leaf of the Ribes host and the type of spore produced, but also between the age of the leaf and the degree of receptivity. Spaulding (*21*) has already stated the above-mentioned facts for *C. ribicola*. Kroemer (*13*) in his investigations of the disease of grape leaves caused by *Plasmopara viticola* reports a somewhat similar connection between the age of the leaves

[6] HAHN, G. G. A PHYSIOLOGICAL METHOD OF DISTINGUISHING CRONARTIUM RIBICOLA AND CRONARTIUM OCCIDENTALE IN THE UREDINIAL STAGE. [Unpublished manuscript.]

and their liability to infection. Under the condition of artificial inoculation he found that young leaves were more vigorously attacked than old ones and that very young leaves completely resisted every attempt at infection.

Observations on greenhouse cultures of both *Cronartium ribicola* and *C. occidentale* showed that they remained viable under favorable conditions for 30 days or more. Other investigators, including Fromme (*9*), Melhus and Durrell (*17*), Doran (*8*), and Peltier (*18*), have shown that the urediniospores of a number of rust species may remain viable for a month or more. Spaulding (*21*) gave the longevity of urediniospores of *C. ribicola* as 7 to 270 days. When inoculations were made under conditions favorable for infection and negative results were obtained, particularly on congenial hosts, there is a possibility that some aberrant condition in the formation or maturation of the urediniospores had interfered with their ability to germinate. A few germination tests with different lots of inoculum indicated that variation in germination does exist. It seems even more likely, however, that some temporary physiological condition of the host existing at the time of inoculation might inhibit infection. This was found to be the case for certain host species during the inoculation experiments when the spores used were known to be viable. As these same plants were successfully inoculated later, the second explanation seems to be the more probable.

METHODS

In general the methods employed in the greenhouse inoculation were in line with those developed by Stakman and his associates (*22*, *23*, *24*), working with cereal rusts, and by Spaulding and his associates (*21*) in the white-pine blister-rust inoculations. Because of cultural differences between the Ribes hosts and such hosts as cereals and grasses, certain necessary modifications in methods were made.

CARE OF RIBES PLANTS TO BE INOCULATED

For the inoculation experiments it was found necessary to insure the production of freshly developed, fully expanded, and unhardened leaves. The cereals and grasses worked upon by other rust investigators may be propagated from seed at any time and are therefore available for inoculation within approximately three weeks. Ribes are not so readily propagated. For these investigations plants of Ribes were secured by the quickest means possible, namely, by growing the cuttings of the vigorous growth of the current season, which were secured in the fall, and by removing plants from the field to the greenhouse where they were grown in pots. In the former case rooted cuttings were usually not available for inoculation purposes until the next year, whereas transplants from the field were frequently usable soon after they were transferred to the greenhouse. In procuring field transplants the tops and roots were cut back, care being taken not to allow the roots to become dry. Shipment was made in slightly moistened moss wrapped in oiled paper. As soon as the plants were received they were potted in good greenhouse soil and placed for a time in a cool situation. Such plants broke into leaf soon after potting, depending, of course, upon the species and the particular adaptiveness of each to such treatment. As a general rule most of the Ribes species responded readily to this treatment.

Ribes plants secured from cuttings varied in that those of some species produced more than one set of leaves for inoculation during the year, and those of others produced only one set of leaves during the season. It was not always possible to secure leaves for inoculation purposes at a specified time. Leaves in condition for inoculation were utilized as they were produced, viable inoculum being provided for the test made under as favorable environmental conditions as possible. Test plants bore metal labels giving not only the name of the host species, but also its source and the date of acquisition. Additional labels gave the data for each inoculation (the date, the species, and the collection number of the fungus strain).

In these investigations it was not possible to discard plants after they had been used once for inoculation purposes, particularly if the plant happened to be a rare species or one difficult to procure or grow. Instead, such plants were stripped of all leaves after results had been recorded, dipped in a corrosive sublimate solution 1:1,000, and set aside in a section of the greenhouse apart from the culture chambers where they could be watched until new leaves appeared. Stock plants for the propagation of inoculum of the different strains were particularly watched for any possible appearance of the rust upon them, but such unintended infections were extremely rare. When the plants were in condition to be reinoculated they were used as hosts for the same rust culture with which they had been previously inoculated. This procedure safeguarded the purity of individual culture strains of the two Cronartiums.

Continual stripping gradually weakened certain of the plants, particularly those difficult to grow in the greenhouse and those with scanty root systems, but vigorous growers such as plants of the *Ribes aureum* group and *R. nigrum* have been defoliated again and again without much apparent injury.

The present investigations were concerned largely with allied species and varieties of the common garden currant, *Ribes sativum* (*R. vulgare*). Inasmuch as this particular host group normally produces only one set of leaves in the spring at the time of fruiting, it became necessary to find a method whereby inoculable leaves could be procured throughout the year, and especially during the late summer and autumn. Plants placed in cold storage before the spring burst, could upon later withdrawal be relied upon to furnish inoculable leaves during the early part of the summer as well as in the late summer and fall, when rust specimens on Ribes from the field are most likely to be sent in for identification.

When plants were not being used in the inoculation experiments they were isolated in sections of the greenhouse where rust cultures were not present, or kept out of doors and brought into the greenhouse at a later date when required. Care was exercised in going from the sections of the house containing plants infected with rust to the rust-free plant sections, so that infection would not be carried over into the latter. To guard against such an occurrence the rust-free plant sections were isolated in inclosed portions of the greenhouse; cloth dusters, which were removed before leaving, were worn in the infected sections, and care was taken at all times to wash the hands thoroughly before handling uninfected plants, particularly after working about rusted ones. Whenever possible, care was exercised in excluding insects from the greenhouse. Inoculations were

never made with the two blister-rust species on the same day. Buffer uninoculated plants of congenial species were maintained on a shelf 5 feet high down the center of the house between the stock cultures on one side bench and the experimental plant inoculation series on the opposite side bench. Througbout the experiments the plants kept on this shelf did not become infected.

MAKING INOCULATIONS

In general, plants were inoculated with spores applied by gently pressing uninfected Ribes leaves that previously had been finely sprayed with water from an atomizer against leaves well covered with fertile spores. Tap water was used with satisfactory results, although Melhus and Durrell (*17*) state that it may have a toxic effect on the germination of certain kinds of rust spores. Recently produced urediniospores were used, as such material usually could be depended upon to germinate to a certain extent. Viable spores were bright orange yellow in color. Care was taken to keep the inoculum from becoming contaminated by mildew or other fungi, which frequently infect greenhouse rust cultures and cause infected areas to die out rapidly, at the same time apparently affecting the viability of the urediniospores themselves. Mildew particularly was troublesome at times, especially in the early spring when the temperature remained low and the sky was overcast. It was kept down to a minimum, however, by picking off and destroying all infected leaves, including those showing the barest trace of mildew. Mildewed leaves rarely became infected, and their inclusion in the inoculations only tended to increase the spread of the contamination.

Plants were also inoculated in the manner described by Carleton (*3*) and Fromme (*9*), that is, by dusting spores from heavy infections over uninfected plants that had been previously sprayed. When the inoculum was very limited, spores were frequently applied to the atomized leaves by means of a scalpel. Extreme care was necessary in this latter process, particularly in the case of very delicate leaves, lest the leaf tissues be injured or killed. Throughout the tests an abundance of inoculating material was highly desirable, in order to insure a general distribution of spores over the leaf surface and maximum infection. Negative inoculation results were confirmed wherever possible by repeating the tests with the same plants and the same rust strains.

TIME OF INOCULATION

Inoculation tests were confined largely to the early spring months, late summer, and autumn. Very little experimental work was conducted during the summer when high temperatures prevailed. However, stock cultures were maintained throughout the high-temperature period. During the summer the glass roof of the greenhouse was lightly whitewashed to cut down the intensity of the sunlight. Iceless refrigerators of the type devised by Hunt (*12*) were used for inoculation, the plants being kept in these moist chambers for 48 hours. During the very warm summer period the inoculation chambers were installed out of doors on the north side of the greenhouse beneath the trees, where the maximum temperature did not exceed 85° F. A continuous flow of water seeping downward through the cloth sides from the water bath on top of the inoculation chamber kept the plants within at a reduced temperature because of the con-

stant evaporation of water from the surfaces of the wet curtains. As in the greenhouse inoculations, the plants were finely sprayed before they were placed within the iceless refrigerator. This film of moisture on the Ribes leaves was constantly maintained while they remained within the inoculation chamber by turning the stream of water from a garden hose quickly and lightly at intervals against the cheesecloth curtains and forcing the water through in a fine spray. Plants inoculated in extremely hot weather were kept out of doors for some days after they were taken from the iceless refrigerator. When pustules began to form the plants were immediately returned to the greenhouse in order to prevent the possible infection of any Ribes that might be in the neighborhood.

CARRYING STOCK CULTURES

Stock cultures of the two Cronartium species were kept in separate compartments of the greenhouse. Partitions of a double thickness of cheesec oth were used in each compartment to separate the side benches into culture chambers in which the individual strains were kept isolated. The cloth partitions extended to the height of the side wall of the house after the manner described by Stakman, Piemeisel, and Levine (*24*). The benches on which the rusted plants stood were kept well drained and fairly dry by covering the greenhouse benches with sifted cinders. An abundant supply of vigorous inoculum was maintained by making new stock cultures approximately every two weeks. The different generations were kept separate. The rusted plants were, moreover, watered only at the base, thereby reducing possible chances for self-inoculation. Generations were indicated on the pot labels by supplementing the original collection number given the strain with abbreviated symbols of the hosts in inoculation sequence. These host abbreviations in turn were supplemented with subnumbers indicating the number of inoculations of each particular Ribes species, i. e., "II *C. ribicola* BC, 38805 nig ^{7} au ^{1}" signifies seven successive generations of uredinia of the British Columbia strain of *Cronartium ribicola* on *Ribes nigrum* followed by one generation on *R. aureum*. A record of the inoculation data of successive generations was kept for future reference in explaining possible variations in the reactions of a given rust strain, particularly with regard to possible decline in vigor.

COMPARATIVE STUDY OF THE TWO CRONARTIUMS

In a series of rust-infection tests, two species of Cronartium were used. The inoculations were made as close to each other in point of time as possible, the procedure being to handle only one species of rust on the same day. More than one strain of the same species, however, was sometimes handled on the same day, but the utmost care was taken to avoid mixtures. Comparative inoculations of the two blister rusts were made wherever possible on Ribes plants of the same species, collection source, and leaf age. Wherever possible both rusts were used on plants derived from the same original parent stock. Occasionally the same plant was inoculated with both rusts. One side or branch of the plant was first inoculated with one of the rusts. During this process the rest of the plant was protected against chance inoculation. Two days later the inoculated part was protected and the opposite side or another branch was inoculated with the other rust.

Throughout the tests an effort was made to maintain environmental conditions influencing rust infection as nearly identical as possible for both rust species. As previously pointed out, these conditions included temperature, humidity, and light, as well as the condition of the leaves of the Ribes host at the time of inoculation. Inoculations were made when possible at temperatures of not more than 75° F. A high relative humidity close to the saturation point was maintained throughout the infection period. After the inoculated test plants were removed from the iceless refrigerator they were placed in the muslin-partitioned isolation chambers on the side benches. Ordinary greenhouse watering was sufficient to carry infected plants to the sporulating stage. The amount of sunlight secured in the greenhouse sufficed for excellent rust development. The light conditions obtained throughout the culture and inoculation test chambers were quite comparable. Records were systematically kept of the first appearance of infection spots, pustules, and spores. These records were complete within 15 days, so far as maximum urediniospore production was concerned. A 15-day period was also found to be sufficient for the appearance of those pathologic symptoms that indicated the particular infection type of susceptibility or resistance of the given host species.

RECORDING DATA

The inoculated plants were classified by infection types, based on the pathologic symptoms produced. Where it was necessary because of a scarcity of good trial-host material to inoculate plants not in condition for inoculation, a record was made at the time of such aberrant host conditions in order to aid in the explanation of possible negative results. An attempt to describe individually all the pathologic symptoms for each tested host species and variety, would have required a great deal of time. Classification symbols were therefore developed, the use of which greatly facilitated the recording of results. The symbols indicating the types of infection are as follows:

Resistant types:

Immune—

○ No uredinia formed; hypersensitive or necrotic areas either definite, indistinct, or entirely lacking.

Resistant—

◑ Uredinia, formed but minute or aborted, surrounded or associated with hypersensitive flecks or larger necrotic areas; flecks in some cases very abundant.

Susceptible type:

● Uredinia, normal size; no hypersensitive or necrotic areas, but in some cases infections surrounded by slightly chlorotic tissue, or uredinia produced on green islands.

The relative quantity or abundance of uredinia produced on the infected leaves was also indicated by symbols. They are as follows:

=, *Trace.*—Uredinia bare trace or very few in number.

−, *Slight infection.*—Number of uredinia below normal, scanty. This symbol is analogous to the single cross, ×, used in former white-pine blister-rust investigations (*21*).

±, *Moderate.*—Medium production of uredinia, average or normal infection. Analogous to the two crosses, ×× (*21*).

+, *Heavy.*—Infection heavier than medium. Analogous to the three crosses, ××× (*21*).

++, *Very heavy.*—Heavy or extremely abundant production of uredinia, covering practically the entire leaf area.

To indicate the number of infected leaves on each plant as related to the number of inoculated leaves, a fraction was used, the denominator giving the number of leaves inoculated and the numerator those showing infection. In recording the abundance of uredinia produced on each host species or variety, a rating was given the plant as a whole.

CORRELATING DATA

To obtain a general figure for the abundance of urediniospores on the uredinia-bearing leaves of a given Ribes species or variety, it seemed best to reduce the symbols described in the preceding section to a numerical basis and average them. Table 1 gives the numerical expression for each symbol.

TABLE 1.—*Mathematical expression of the abundance of uredinia production*

Symbols for relative abundance of uredinia on infected leaves	Equivalent symbols	Range of class [a]	Mid-value of class [a]	Abundance of uredinia
		Per cent	*Per cent*	
=	(×)	Less than 5	2.5	Trace.
−	[b] ×	5–35	20.0	Slight.
±	[b] ××	35–65	50.0	Moderate.
+	[b] ×××	65–85	75.0	Heavy.
+ +		85–100	92.5	Very heavy.

[a] The percentage values are rational expressions of the abundance of uredinia production based on the maximum uredinia development on completely infected leaves of *Ribes nigrum*, under favorable conditions, which were taken as a standard. In converting the symbols into numbers, each was assigned the mid-value of the class it represents.

[b] Used in previous white pine blister-rust investigations. See Spaulding (*21*). Range percentages and values of classes do not apply to these symbols.

In Figures 1 to 3 the class ranges are indicated by horizontal lines. In obtaining the average abundance values shown by the broken line in Figures 1 and 2 and by both lines in Figure 3, the rating for each plant was weighted by the number of spore-producing leaves on the plant.

In Figures 1 and 2 the index of the abundance of uredinia per unit area of all infected leaves for each species is shown by a cross (×) without connecting lines. This was obtained by multiplying the converted mathematical value of the average abundance on uredinia-bearing leaves by the percentage of inoculated leaves that produced fertile uredinia, and dividing the product by 100.

The foregoing classification of infection types and abundance of uredinia for the two rusts, *Cronartium ribicola* and *C. occidentale*, follows that laid down by Vavilov (*25*), who in his investigations of grain rust in the field utilized a classification similar to that employed by Eriksson, which was based on the total production of uredinia and the character and development of the lesions. Stakman and Levine (*22*) adopted a system that is very similar to that of Vavilov. Melhus, Dietz, and Willey (*16*), working with crown rust, *Puccinia coronata*, classified the rust infections they obtained into only three infection types. Their types were arbitrarily chosen to state differences not fixed, but including a certain amount of variation in the degree of infection between the two extremes in each type. The system chosen for the present problem is similar to theirs, in that only three infection types are distinguished, but it differs in that the production of uredinia is considered as a separate though closely

associated criterion. The infections were not separated into as many types as were employed by Stakman and Levine (*22*), for the reason that good division points could not be obtained for so many. With the system employed there was rarely any serious doubt as to the class to which an infection should be assigned. The use of narrower and more numerous classes would have made assignment difficult.

The recognition of small differences in reactions of the Ribes hosts to the parasite is more difficult than with the grain rusts, both because there are so few distinguishable types on Ribes and because of the heterogeneity of the host material. The grain and bean hosts on which comparisons have been made have been mainly

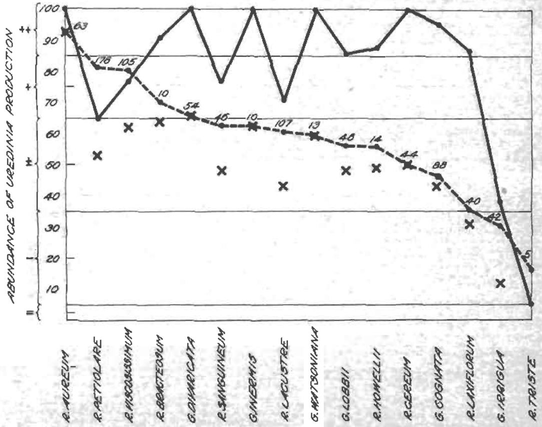

FIG. 1.—Results of inoculations with *Cronartium ribicola* on Ribes and Grossularia plants received from the Pacific Northwest. Only the converted mathematical value of the average abundance of uredinia produced on leaves on which at least some fruiting occurred is considered. Solid line: Percentage of inoculated leaves that produced fertile uredinia. Broken line: The average abundance of uredinia per unit area of leaves on which at least some fertile uredinia were formed. The figures above the points show the total number of such leaves. The symbols at the left margin and their conversion to an arithmetical basis is explained on p. 671. × (points not connected by lines): An index of the abundance of uredinia produced per unit area of leaf inoculated; obtained as described on p. 672

pure lines. The Ribes material from the Northwest consisted of native plants, which are presumably highly heterozygous.

Wherever possible, abundant specimens, which will be permanently available for reference, were collected for the herbarium to supplement the notes taken. When dried quickly under moderate pressure these specimens did not lose much in the way of color or other characteristics.

HOST PLANTS TESTED

The geographical group of native Grossulariaceae treated in this paper, namely, a Pacific northwestern group, which has been classified

by Wyckoff as properly belonging in a key for Ribes of Washington,[7] is of particular interest at this time, in that it involves species of Ribes and Grossularia that occur in the regions of advance infections of the introduced *Cronartium ribicola* in the Northwest and which are or may be native hosts of *C. occidentale*. The group includes: *R. bracteosum* Dougl.; *R. petiolare* Dougl. (*R. hudsonianum petiolare* Jancz.); *R. howellii* Greene; (*R. acerifolium* Howell); *R. sanguineum* Pursh; *R. laxiflorum* Pursh; *R. aureum; R. triste* Pall. (*R. ciliosum* Howell;

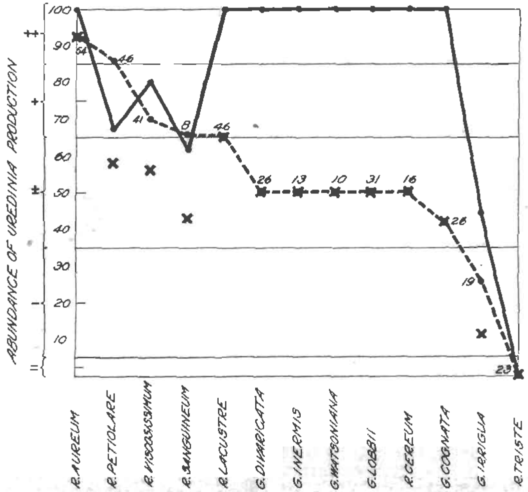

FIG. 2.—Results of inoculations with *Cronartium occidentale* on Ribes and Grossularia plants received from the Pacific Northwest. Only the converted mathematical value of the average abundance of uredinia produced on leaves on which at least some fruiting occurred is considered. Solid line: Percentage of inoculated leaves that produced fertile uredinia. Broken line: The average abundance of uredinia per unit area of leaves on which at least some fertile uredinia were formed. The figures above the points show the total number of such leaves. The symbols at the left margin and their conversion to an arithmetical basis is explained on p. 671. × (points not connected by lines): An index of the abundance of uredinia produced per unit area of leaf inoculated; obtained as described on p. 672

R. migratorium Suksd.); *R. cereum* Dougl. (*R. inebrians* Lindl. or *R. reniforme* Nutt.); *R. viscosissimum* Pursh; *R. lacustre* (Pers.) Poir. (*R. parvulum* Rydb.); *R. montigenum* McClatchie (*R. molle* Howell, *R. lentum* Coville and Rose); *Grossularia lobbii* (A. Gray) C. and B. (*R. lobbii* A. Gray); *G. watsoniana* (Koehne) C. and B. (*R. watsonianum* Koehne, *R. ambiguum* S. Wats., in part); *G. cognata* (Greene) C. and B. (*R. cognatum* Greene); *G. irrigua* (Dougl.) C. and B. (*R. irriguum* Dougl., *R. divaricatum irriguum* A. Gray, *R. leucoderme* Heller); *G.*

[7] WYCKOFF, S. N. THE RIBES OF WASHINGTON. 39 p., illus. (U. S. Dept. Agr., Bur. Plant Indus., Off. Blister Rust Control.) 1922. [Mimeographed.]

nivea (Lindl.) Spach. (*R. niveum* Lindl.); *G. divaricata* (Dougl.) C. and B. (*R. divaricatum* Dougl., *R. villosum* Nutt., *R. suksdorfii* Heller); and *G. inermis* (Rydb.) C. and B. (*R. inerme* Rybd., *R. purpusi* Koehne, *R. vallicola* Greene).

All of these species, which were determined in the field, but which did not flower in the greenhouse and thus make further determination possible, were inoculated with *Cronartium ribicola* with the exception of *Ribes montigenum* and *Grossularia nivea*, plants of which were not

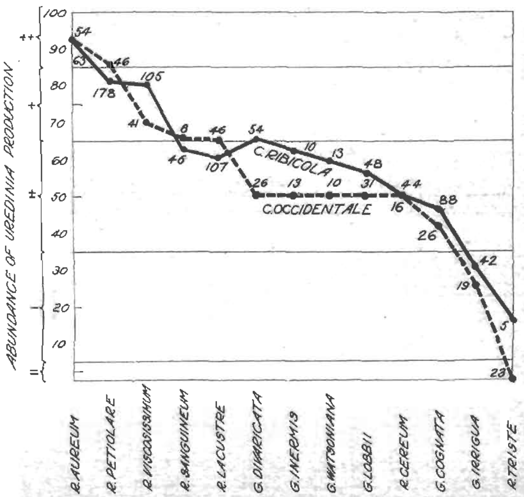

FIG. 3.—Comparison of results of inoculations with *Cronartium occidentale* and *C. ribicola* on Ribes and Grossularia plants as expressed in Figures 1 and 2. Only the average abundance of uredinia on the leaves on which at least some uredinia were formed is considered. The Ribes species that were inoculated with both rusts are included; they are arranged in descending order of the abundance of the uredinia production for *C. occidentale*

available for inoculation purposes. For the same reason these two species and *R. bracteosum*, *R. howellii*, and *R. laxiflorum* were not inoculated with *C. occidentale*.

RESULTS

All the species of the Ribes and Grossularia tested, irrespective of source, proved susceptible to *Cronartium ribicola*, though some were much less congenial hosts than others. Likewise, practically all proved to be more or less susceptible to *C. occidentale*, except one species, *R. triste*, which was immune. This Ribes species was only slightly susceptible to *C. ribicola*.

TABLE 2.—*Results of inoculating species of Ribes and Grossularia with Cronartium ribicola*

[Types of infection: ○, immune; ◑, resistant; ●, susceptible. Relative abundance of uredinia: =, trace; −, slight; ±, moderate; +, heavy; and ++, very heavy]

Species inoculated		Number of plants inoculated	Leaves inoculated		Number of plants with indicated percentages of inoculated leaves producing uredinia				Number of plants showing on their spore-producing leaves the indicated abundance of uredinia					Number of plants of specified infection type		
Name	Source		Number	Per cent infected	0	1–49	50–99	100	=	−	±	+	++	○	◑	●
Ribes aureum	Idaho (Marble Creek)	6	63	100	0	0	0	6	0	0	0	0	6	0	0	6
Ribes petiolare	Idaho (Elk River)	6	34	29	4	0	0	2	0	0	0	0	2	4	0	2
	Idaho (Marble Creek)	27	205	82	2	0	10	15	1	2	3	0	19	2	3	22
	Utah (Salt Lake City)	4	36	0	4	0	0	0						4	0	0
Total		37	275	65	10	0	10	17	1	2	3	0	21	10	3	24
Ribes viscosissimum	Washington (Yakima)	9	51	90	0	1	1	7	1	0	1	0	7	0	0	9
	Idaho (Hayden Lake)	1	7	86	0	0	1	0	0	0	1	0	0	0	0	1
	Idaho (Elk River)	6	33	79	2	0	0	4	0	1	0	0	3	2	0	4
	Idaho (Sandpoint)	2	28	43	1	0	0	1	0	0	0	0	1	1	0	1
	Idaho (Elbe River)	2	18	83	0	0	2	0	0	1	0	1	0	0	0	2
Total		20	137	77	3	1	4	12	1	2	2	1	11	3	0	17
Ribes bracteosum	Washington (Hoquiam)	1	5	80	0	0	1	0	0	0	1	0	0	0	0	1
	Washington (Aberdeen)	2	6	100	0	0	0	2	0	0	0	1	1	0	0	2
Total		3	11	91	0	0	1	2	0	0	1	1	1	0	0	3
Grossularia divaricata	British Columbia (Vancouver)	6	54	100	0	0	0	6	0	0	3	3	0	0	0	6
Ribes sanguineum	do	9	60	77	0	3	2	4	2	1	4	0	2	0	2	7
Grossularia inermis	Washington (Yakima County)	1	5	100	0	0	0	1	0	0	1	0	0	0	0	1
	Idaho	1	5	100	0	0	0	1	0	0	0	1	0	0	0	1
Total		2	10	100	0	0	0	2	0	0	1	1	0	0	0	2
Ribes lacustre	British Columbia (Stanley Park)	2	14	50	0	1	0	1	1	1	0	0	0	0	0	2
	Idaho (Moscow)	17	137	73	3	1	3	10	0	1	6	7	0	3	0	14
Total		19	151	71	3	2	3	11	1	2	6	7	0	3	0	16

Grossularia watsoniana	Washington (Yakima)	1		100	0	0	0	1	0	0	0	1	0	0	0	1
	Idaho (Moscow)	1	8	100	0	0	0	1	0	0	1	0	0	0	0	1
Total		2	13	100	0	0	0	2	0	0	1	1	0	0	0	2
Grossularia lobbii	British Columbia (Vancouver)	4	56	86	1	0	0	3	0	0	2	1	0	1	0	3
Ribes howellii	Washington (Lake Keechelus)	5	16	88	2	0	0	3	0	0	2	0	1	2	0	3
Ribes cereum	Washington (Spokane)	4	44	100	0	0	0	4	0	0	4	0	0	0	0	4
Grossularia cognata	Idaho (St. Joe)	8	74	100	0	0	0	8	1	0	4	3	0	0	0	8
	Idaho (Sandpoint)	2	18	78	0	0	2	0	1	0	1	0	0	0	0	2
Total		10	92	96	0	0	2	8	2	0	5	3	0	0	0	10
Ribes laxiflorum	Washington (Yakima)	1	3	100	0	0	0	1	0	0	1	0	0	0	0	1
	Washington (Aberdeen)	8	43	86	0	0	4	4	1	3	4	0	0	0	0	8
Total		9	46	87	0	0	4	5	1	3	5	0	0	0	0	9
Grossularia irrigua	Idaho (Oxford)	9	71	13	5	3	1	0	1	3	0	0	0	5	4	0
	Idaho (Moscow)	7	38	87	1	0	1	5	0	3	3	0	0	1	3	3
Total		16	109	39	6	3	2	5	1	6	3	0	0	6	7	3
Ribes triste	British Columbia (Vancouver)	2	3	0	2	0	0	0						2	0	0
	Idaho (Moscow)	16	71	7	14	1	0	1	1	1	0	0	0	14	0	2
	Washington (Yakima)	7	19	0	7	0	0	0						7	0	0
Total		25	93	5	23	1	0	1	1	1	0	0	0	23	0	2

TABLE 3.—*Results of inoculating species of Ribes and Grossularia with Cronartium occidentale*

[Types of infection: ○, immune; ◑, resistant; ●, susceptible. Relative abundance of uredinia: =, trace; −, slight; ±, moderate; +, heavy; and ++, very heavy]

Species inoculated: Name	Species inoculated: Source	Number of plants inoculated	Leaves inoculated: Number	Leaves inoculated: Per cent infected	Number of plants with indicated percentages of inoculated leaves producing uredinia: 0	1–49	50–99	100	Number of plants showing on their spore-producing leaves the indicated abundance of uredinia: =	−	±	+	++	Number of plants of specified infection type: ○	◑	●
Ribes aureum	Idaho (Marble Creek)	5	54	100	0	0	0	5	0	0	0	0	5	0	0	5
Ribes petiolare	do	10	58	79	2	0	2	6	0	0	1	1	6	2	0	8
	Utah (Salt Lake City)	1	10	0	1	0	0	0	...	...	...	...	...	1	0	0
Total		11	68	68	3	0	2	6	0	0	1	1	6	3	0	8
Ribes viscosissimum	British Columbia (Vancouver)	1	7	100	0	0	0	1	0	0	0	0	1	0	0	1
	Oregon (Mount Hood)	2	11	82	0	0	1	1	0	0	1	0	1	0	0	2
	Idaho (Hayden Lake)	1	4	100	0	0	0	1	0	0	1	0	0	0	0	1
	Idaho (Elk River)	2	13	100	0	0	0	2	0	0	0	0	2	0	0	2
	Idaho (Sandpoint)	2	16	50	1	0	1	0	0	1	0	0	0	1	1	0
Total		8	51	80	1	0	2	5	0	1	2	0	4	1	1	6
Ribes sanguineum	British Columbia (Vancouver)	3	13	62	0	0	1	2	0	1	1	0	1	0	0	3
Ribes lacustre	Idaho (Moscow)	5	46	100	0	0	0	5	0	0	2	3	0	0	0	5
Grossularia divaricata	British Columbia (Vancouver)	3	26	100	0	0	0	3	0	0	3	0	0	0	0	3
Grossularia inermis	Washington (Yakima)	2	13	100	0	0	0	2	0	0	2	0	0	0	0	2
Grossularia watsoniana	Idaho (Moscow)	1	10	100	0	0	0	1	0	0	1	0	0	0	0	1
Grossularia lobbii	British Columbia (Vancouver)	3	31	100	0	0	0	3	0	0	3	0	0	0	0	3
Ribes cereum	Washington (Spokane)	2	16	100	0	0	0	2	0	0	2	0	0	0	0	2
Grossularia cognata	Idaho (Sandpoint)	4	26	100	0	0	0	4	0	1	3	0	0	0	0	4
Grossularia irrigua	Idaho (Oxford)	5	27	11	4	1	0	0	1	0	0	0	0	4	0	1
	Idaho (Moscow)	4	16	100	0	0	0	4	1	1	2	0	0	0	0	4
Total		9	43	44	4	1	0	4	2	1	2	0	0	4	0	5
Ribes triste	Idaho (Moscow)	5	23	0	5	0	0	0	...	...	...	...	...	5	0	0

In the work with the northwestern species 1,230 leaves on 177 plants were inoculated with *Cronartium ribicola*, and 420 leaves on 61 plants with *C. occidentale*. The results of these inoculations are shown in detail in Tables 2 and 3 for the two blister rusts, respectively.

The host material, which is listed in Table 2, does not actually represent the entire distribution of each species from the Pacific Northwest. The representation, however, is to a certain degree indicative for each species; for, despite certain exceptions in species from other regions, it has been the experience of the writer that plants of the same Ribes species from various geographical sources generally react uniformly with respect to rust infection. Some of the plants listed in the table were grown from cuttings received from field men. In such cases it is not known whether each cutting was from a different bush or whether all the cuttings from a particular locality were taken from the same bush. The total number of original plants represented by the inoculated plants is thus probably somewhat less than the total of the figures in column 3 of Table 2.

All the plants inoculated are included in Table 2 irrespective of their leaf condition. Because of scarcity of certain host material it was sometimes necessary to use plants that were not in optimum condition for inoculation purposes. Mildewed or hardened leaves in certain of the tests with *Ribes petiolare*, *R. viscosissimum*, *R. lacustre*, *Grossularia lobbii*, *R. howellii*, and *R. triste* gave negative results.

The results for all the inoculations with *Cronartium occidentale* are given in Table 3. The host species used were obtained and utilized as explained above. To make the comparison between the two Cronartiums as direct as possible, the same plant or plants were regularly used for both rusts in inoculation tests made at different times. Comparisons obtained on identical plants in this way corroborated other results obtained on different plants from the same geographical source. As in the case of *C. ribicola*, no difference was observed in the way in which plants of the same Ribes species but from different parts of the Northwest reacted toward *C. occidentale*.

In the case of very young leaves inoculated just below the terminal or growing-shoot tip, infections of the resistant type were encountered with *Ribes viscosissimum*, *R. petiolare*, *R. howellii*, *R. laxiflorum*, *R. lacustre*, and *R. sanguineum*. Undersized uredinia associated with flecks or larger necrotic areas on the immature leaves of these species gave place to normal uredinia on further developed and fully expanded leaves. On *Grossularia irrigua*, however, a considerable percentage of leaves were either immune or resistant, the latter producing a trace or scant number of aborted pustules associated with flecks and necrosis. This species produced a low percentage of fully susceptible leaves. Leaves of *R. triste* showed a marked tendency to become infected with *Cronartium ribicola* for only a very limited period. Very young leaves produced flecks only, or no signs of infection at all, and fully expanded leaves appeared to become quickly resistant; those that had apparently started to undergo the hardening process were quite immune. Of all the Northwest species handled, *R. triste* appeared to be most difficult to propagate in a healthy condition. This species was particularly susceptible to mildew, which greatly interfered with the results of the rust inoculations.

The different grades of abundance of uredinia production were assigned arithmetical values on a scale of 100, as explained on page 10. The averages of these values for each host species are shown graphically in Figures 1, 2, and 3. In preparing these graphs the results on host material that was not in good condition for inoculation have been eliminated. In reporting the abundance of uredinia production only the infected leaves are considered. The product of this abundance value and the percentage of leaves infected is perhaps a better indication of the amount of uredinia production that might be expected under field conditions. These products for the different Ribes species are shown in Figures 1 and 2 by crosses (×) with no connecting lines.

Some of these greenhouse inoculation results for *Cronartium ribicola* corroborate those already published by Spaulding (*21*). Additional results are given for the first time for native northwestern Ribes which were not available to him, namely, *Ribes petiolare*, *R. howellii*, *R. laxiflorum*, *Grossularia watsoniana*, and *G. cognata*. Artificial inoculations with *C. occidentale* have been previously reported on *R. aureum*, *G. inermis* (*11*), and *G. divaricata* (*4*). All the artificially inoculated hosts of the piñon rust investigated in this paper, other than the three species just named, are therefore reported for the first time.

PACIFIC NORTHWEST RIBES GENERALLY SUSCEPTIBLE TO CRONARTIUM RIBICOLA

The group of northwestern Ribes susceptible to *Cronartium ribicola* presented in Figure 1 are arranged in descending order of abundance of uredinia production. *Ribes aureum* was found generally to harbor a very heavy infection. *R. petiolare*, *R. viscosissimum*, *R. bracteosum*, and *Grossularia divaricata* showed heavy infections. The last-named host was approximately on the class-range boundary line separating the heavy from the moderate infections. On more than half the host species a moderate number of uredinia was produced. Only a scant infection occurred on *G. irrigua* and *R. triste*.

So far as the conclusions from the results of artificial inoculation in the greenhouse can be applied to the field, it is very evident from the data presented in Table 2 and Figure 1 that there is practically no hope of the Idaho white-pine forests escaping the white-pine blister rust because of immunity of the local Ribes species.

PACIFIC NORTHWEST RIBES GENERALLY SUSCEPTIBLE TO CRONARTIUM *OCCIDENTALE*

The northwestern Ribes group susceptible to *Cronartium occidentale* presented in Figure 2 are arranged in descending order of congeniality to the piñon rust. *Ribes aureum*, a favorite host for *C. occidentale*, was constantly and very heavily infected. Heavy infections were secured on *R. petiolare*, *R. viscosissimum*, and *R. lacustre;* scanty infections were produced on *Grossularia irrigua*; *R. triste* remained quite immune. About half the species belonged in the moderately infected class.

PHYSIOLOGICAL SIMILARITY BETWEEN THE TWO CRONARTIUMS ON NORTHWEST RIBES

From a consideration of Figures 1 and 2 it is very evident that the abundance-of-uredinia production curves for *Cronartium ribicola* and for *C. occidentale* on the northwestern Ribes follow each other rather

closely. The parallelism between the results with the two different species of blister rust is indeed very striking, showing how great a physiological similarity with regard to host preference exists between them. This fact, added to their morphological resemblance, impresses on one how slight are the actual differences that exist between these two Cronartiums in the uredinial stage. Figure 3 brings out this physiological similarity between the two blister rusts.

VALUE OF THE NORTHWESTERN SPECIES AS DIFFERENTIAL HOSTS FOR DISTINGUISHING THE TWO RUSTS

From a consideration of the physiological reaction of the two Cronartiums on species of Pacific Northwest Ribes, it is very evident that only one species could possibly be used as a differential host. This species, *R. triste,* proved slightly susceptible to *Cronartium ribicola* and immune to *C. occidentale.* The tests were few and on leaves in poor condition. Because of the difficulties encountered in the propagation and maintenance of this wild host species under greenhouse conditions and its uncertain reaction to *C. ribicola,* it is certainly less useful as a differential host than are a number of the cultivated varieties of its close relative, the common red garden currant, *R. sativum* (*R. vulgare*).

SUMMARY

Species of all the Pacific northwestern Ribes obtainable were inoculated with *Cronartium ribicola* and *C. occidentale* in a greenhouse at Washington, D. C. The methods used in inoculating the Ribes and in determining the results of inoculation are described in detail. The demands of the Ribes host made necessary a considerable modification of the methods used by investigators of cereal rusts in recording and correlating data.

Essential physiological differences were not discovered between the two Cronartiums in the uredinial stage under artificial greenhouse conditions, except in one case. *Ribes triste,* the wild relative of the cultivated *R. sativum* (*R. vulgare*), was immune to *Cronartium occidentale* and susceptible to *C. ribicola.* In these experiments *R. triste* appeared to be a host which was only slightly susceptible to the latter rust, which circumstance, together with difficulties of greenhouse propagation, probably renders this species of little value as a differential host.

Practically all of the northwestern Ribes species proved decidedly susceptible to *Cronartium ribicola.* The inoculation results, taken together with what is known of Ribes distribution in the Northwest eliminate any chance that the *Pinus monticola* forests will escape white-pine blister rust because of the lack of susceptible alternate hosts.

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